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# Production of a SARS-CoV-2 Spike Protein Vaccine Using the Baculovirus Expression Vector System

Rachel Adler

Nathan Kelsey

Michelle Maik

Jun Ho Song

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## Abstract

Various COVID-19 vaccines are currently in development, as the COVID-19 pandemic has created an unmet need for protection against the SARS-CoV-2 virus. While there are many different types of vaccines, we focused on developing one that would be safe, affordable, and quickly available for emergency use. A vaccine synthesized using recombinant proteins utilizes a reliable and well-studied technological platform, avoids the safety risks inherent to viral vectors, and provides a cost-effective, scalable method of production of antigen used to induce an immune response. Other vaccines on the market notably include Pfizer's and Moderna's mRNA based vaccines. Although these are widely used, there is still a large demand for an inexpensive yet safe and effective vaccine. Herein, we propose the production of 500 million doses of a recombinant spike protein-based COVID-19 vaccine in a quick time frame and cost-effective manner, using the baculovirus expression vector system (BEVS). Our upstream process involves a three-stage cellular scale-up from shake flasks to WAVE bioreactors to perfusion to production bioreactors, as well as an additional two-stage viral amplification from flasks to WAVE bioreactors. Our downstream process involves a six-stage protein recovery with depth filtration, his-tag chromatography, viral inactivation, ion-exchange chromatography, viral filtration, and diafiltration. We will be partnering with a contract manufacturing organization (CMO) for this project, as we do not have the time to quickly build a plant to get these vaccines out for emergency use. This arrangement makes this process highly profitable. Selling each dose for $16 yields net earnings near $2 billion and an extremely high IRR due to the lack of permanent and fixed costs other than our rental fee. The IRR for the CMO is estimated to be at least 16% with the NPV of the plant at $855,000 and an ROI of 18%.



## Disciplines

Biochemical and Biomolecular Engineering | Chemical Engineering | Engineering

# Letter of Transmittal

University of Pennsylvania, School of Engineering and Applied Science
Department of Chemical and Biomolecular Engineering
220 South 33rd Street
Philadelphia, PA 19104

April 20, 2021

Dear Dr. Miriam Wattenbarger, Prof. Bruce Vrana, and Dr. Jeffrey Cohen,

This report proposes a process design that produces a recombinant spike-protein-based COVID-19 vaccine. The final drug substance recovered contains 2.60 kg of highly purified protein, sufficient for more than the 500 million doses requested in the original project statement. The process design involves scale-up of cells from shake flasks to bioreactors, viral amplification, and protein recovery through steps including filtration, viral inactivation, and chromatography. This design ensures a product that is both safe and affordable for consumers, while easily replicable for the CMO to manufacture.

An economic analysis of this process shows favorable profitability. By partnering with a CMO in North Carolina, the manufacture of SARS-CoV-2 spike protein-based vaccine brings in $2 billion in net earnings at a net present value of $1.5 billion. The CMO plant has a return on investment of 18%, an investor's rate of return of at least 16%, and a net present value of $855,000. These numbers suggest that the project is profitable, and a CMO would respond favorably to a manufacturing partnership. We recommend that the design move forward following the specifications in this report. The economics can be supplemented by considerations of research and development investments and formulation and packaging costs, which were outside the scope of this project.

Thank you so much for all the assistance with this senior design project. We greatly appreciate your guidance in this unprecedented Spring 2021 semester amidst COVID-19.

Sincerely,

Rachel Adler Nathan Kelsey Michelle Maik Jun Ho Song

# Production of a SARS-CoV-2 Spike Protein Vaccine Using the Baculovirus Expression Vector System

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Rachel Adler
Nathan Kelsey
Michelle Maik
Jun Ho Song

Project Author: Dr. Jeffrey D. Cohen
Project Advisor: Dr. Miriam Wattenbarger

University of Pennsylvania
School of Engineering and Applied Science
Department of Chemical and Biomolecular Engineering
April 20, 2021

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# 1. Abstract

Various COVID-19 vaccines are currently in development, as the COVID-19 pandemic has created an unmet need for protection against the SARS-CoV-2 virus. While there are many different types of vaccines, we focused on developing one that would be safe, affordable, and quickly available for emergency use. A vaccine synthesized using recombinant proteins utilizes a reliable and well-studied technological platform, avoids the safety risks inherent to viral vectors, and provides a cost-effective, scalable method of production of antigen used to induce an immune response. Other vaccines on the market notably include Pfizer's and Moderna's mRNA based vaccines. Although these are widely used, there is still a large demand for an inexpensive yet safe and effective vaccine. Herein, we propose the production of 500 million doses of a recombinant spike protein-based COVID-19 vaccine in a quick time frame and cost-effective manner, using the baculovirus expression vector system (BEVS). Our upstream process involves a three-stage cellular scale-up from shake flasks to WAVE bioreactors to perfusion to production bioreactors, as well as an additional two-stage viral amplification from flasks to WAVE bioreactors. Our downstream process involves a six-stage protein recovery with depth filtration, his-tag chromatography, viral inactivation, ion-exchange chromatography, viral filtration, and diafiltration. We will be partnering with a contract manufacturing organization (CMO) for this project, as we do not have the time to quickly build a plant to get these vaccines out for emergency use. This arrangement makes this process highly profitable. Selling each dose for $16 yields net earnings near $2 billion and an extremely high IRR due to the lack of permanent and fixed costs other than our rental fee. The IRR for the CMO is estimated to be at least 16% with the NPV of the plant at $855,000 and an ROI of 18%.

# 2. Introduction and Objective-time Chart

## 2.1 Project Background

The COVID-19 global pandemic has been caused by the rapid global transmission of SARS-CoV-2, a novel coronavirus that was first reported in December 2019 following an initial outbreak in Wuhan, Hubei Province, China [1]. As of March 28, 2021, there have been over 126 million confirmed cases and nearly 2.8 million deaths worldwide [2]. In the United States alone, there have been over 30 million cases and nearly 550,000 deaths [3]. These numbers stress the urgent need for vaccine development, manufacture, and global distribution.

Vaccines are designed to help the human body build an immune response to a particular pathogen. By inducing an immunogenic response, the body can respond to any future infection by minimizing or eliminating symptoms and/or the disease itself. In developing vaccines, scientists consider the immune response, the target population, and the best available technology. This process normally takes years: it took 16 years to develop a hepatitis B vaccine and 5 years to develop an Ebola vaccine [4].

Due to the urgency of the pandemic, there is immense pressure to develop a vaccine against SARS-CoV-2 within 18 months. In addition to accelerated vaccine development, the quick scale-up of a viable manufacturing and purification process is crucial to producing large quantities of vaccines to meet global demand. Previous research, such as that for SARS-CoV vaccine development [5] has established the spike protein of SARS-CoV-2 as a clear target candidate for vaccines [1].

## 2.2 Project Goals and Scope

The goal of this project will be to select a vaccine technology and design both an upstream and downstream manufacturing process to produce 500 million doses of a COVID-19 vaccine. Of the available vaccine technologies, the traditional live-attenuated and inactivated virus vaccines, which use the weakened or killed virus, respectively, were deemed too dangerous due to their usage of actual SARS-CoV-2 virus. The more recent mRNA, viral vector, and subunit vaccine technologies will be considered and discussed in Section 10.1 [6][7].

The upstream process will consist of a series of flasks and bioreactors used to grow a large population of cells, followed by inoculation of the final cell population with viral material to produce the desired active pharmaceutical ingredient (API). The subsequent downstream process will include a series of unit operations to isolate, purify, and package the bulk API. The genetic modification of the cell line and viral material, and the formulation and packaging of the API into doses is outside the scope of this project. The capital investment and operating costs for the upstream and downstream processes will be estimated to price the production of a large volume of vaccine. This economic analysis will allow the team to approach a CMO with the available equipment and resources to negotiate a reasonable contract. The urgency of the COVID-19 pandemic necessitates that vaccines be produced as soon as possible by a capable CMO as opposed to the traditional design of a new manufacturing facility.

## 2.3 Objective-Time Chart

*Figure 2.1* shows the established timeline the team followed in completing this project. In January, we met with project author Dr. Jeffrey Cohen and faculty advisor Dr. Miriam Wattenbarger to discuss and review the literature available. This research and their expertise informed and guided our preliminary process synthesis of the manufacturing process in late January and early February. The month of February was dedicated to designing the overall upstream (i.e. harvest) and downstream (i.e. purification) processes. The team also began writing the report mid-February to document information and decisions made along the way. The end of February and the month of March focused on the specific design of the equipment used in each unit operation. The first week of March was used to present our progress since the beginning of the project and the next steps to be taken. The rest of March emphasized the market and economic aspects of costing and profitability analysis. In the first half of April, the process flow diagrams (PFDs), material balances, energy balances, and profitability analysis were completed to wrap up the project and finish the written report. Afterwards, the written report and presentation were revised and finalized.

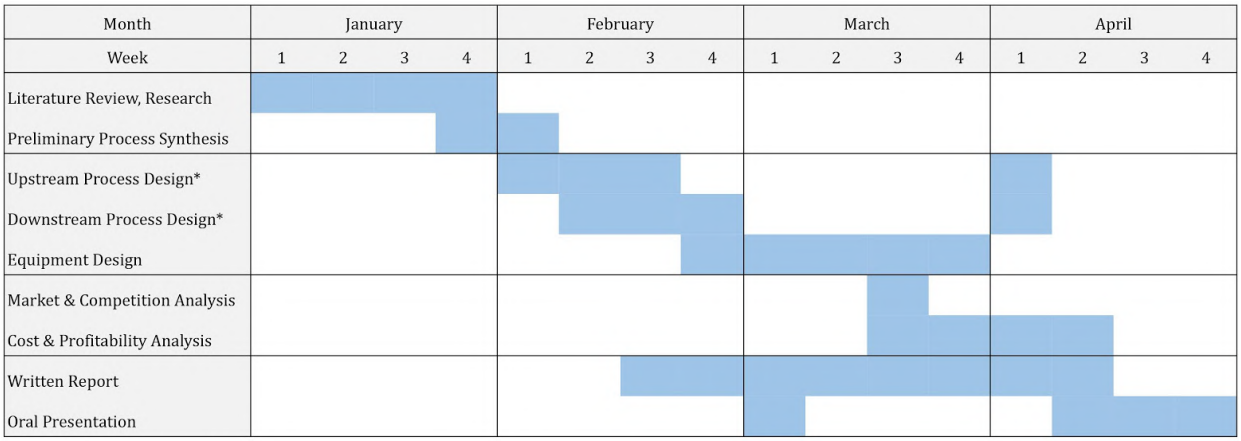


Figure 2.1. Objective-Time Chart for the COVID-19 Vaccine Manufacturing Process Project.

# 3. Innovation Map

**N/A**

# 4. Market and Competitive Analysis

The market demand for SARS-CoV-2 vaccines remains very high. With over 1 million total cases, and the number of new cases still averaging about 500,000 a day, the vaccine is clearly essential. This global pandemic has weakened our health systems, economic systems, and social progression as a whole. In order for businesses and schools to reopen, it is key that as many people as possible are vaccinated against this virus. The global vaccine market revenue is currently estimated at $59 billion USD [8]. By the end of 2021, some have predicted that the market will grow to be worth $75 billion USD [9]. Evidently, there is a huge need for COVID-19 vaccines and our manufacturing process can help to fill this gap.

There may be an initial drop in 2022 because boosters may not be needed, and there may be a price reduction due to intense competition. However, as years pass, the market is expected to open and grow again due to an unmet need for vaccine boosters [8]. The market is subsequently expected to grow at a compound annual growth rate (CAGR) of over 9%, and reach $47.5 billion USD by 2026.

Currently, the market is segmented by technology and geography. Many types of vaccines are in development, as highlighted later in Section 10.1. Additionally, the US, UK, India, Spain, Italy, and China were some of the most affected countries. Currently, 58% of the vaccine market belongs to APAC countries, 15% to Europe, and 12% to North America [10]. However, North America's share of the market is expected to increase to about 46% [9].

There are some pharmaceutical companies who have already begun manufacturing and distributing a vaccine. Notably, Pfizer/BioNTech, Moderna, AstraZeneca/Oxford, Novavax, and GSK are among the largest players. Pfizer/BioNTech agreed to provide the UK with 500 million doses by the end of 2021 [9]. In total, over 1 billion doses of Pfizer/BioNTech's vaccine have

been ordered. Topping this, 3 billion doses from AstraZeneca/Oxford's vaccine, 1 billion doses from Novavax's vaccine, and 800 million doses from Moderna's vaccine have been ordered. Others include Johnson & Johnson's vaccine which has received 300 million dose orders [11].

With almost 8 billion people in the world and most vaccines requiring two doses, there is clearly a large unmet need for vaccines. Our vaccine only requires refrigeration, as opposed to the mRNA vaccines that require frozen storage, which low resource countries do not have the infrastructure for. As of March 24th, about 300 million people worldwide have received at least one dose of a vaccine [12]. This only corresponds to 3.56% of the world's population. Thus, assuming at least 50% of the population will want a vaccine, billions more doses are necessary. Our project aims to produce 500 million doses of a vaccine to contribute to about 250 million complete vaccinations. While competition undoubtedly exists, the most important thing right now is to ensure that as many people as possible are vaccinated as soon as possible. Our project provides a safe, effective, low cost, easily scalable, and highly competitive manufacturing process.

# 5. Customer Requirements

## 5.1 Customer Analysis

The magnitude and swiftness of the COVID-19 pandemic presents a problem of unique proportions in both a public health and economic sense. Customers are effectively the entire global population, particularly the most vulnerable members of society. Since the vaccine is injected into the human body, extra precaution must be taken to ensure that the vaccine is completely safe. Additionally, the refrigeration requirements of our vaccine provide availability to low resource countries that lack cold storage for mRNA vaccines. A vaccine presents the means of stopping the spread, protecting the vulnerable population, and enabling an economic reopening. This occurs when herd immunity is achieved [13]. Therefore, the sooner sufficient vaccination is carried out, the better.

## 5.2 Product Safety

The vaccine must meet stringent safeguards in accordance with Good Manufacturing Practices (GMP) to obtain FDA approval [14]. Therefore, the first priority of this process is to ensure that a product is of utmost safety and purity. Accordingly, this proposal prioritizes purity rather than yield for downstream processes and ensures adequate purification steps to reduce viral content and other contaminants present in the process. Additionally, the upstream process incorporates safety measures such as replicate batches with independent supply lines and media filters to adhere to quality control standards.

## 5.3 Rapid Timeline

Safe but rapid delivery of the vaccine dosage will be critical in limiting the spread of COVID-19. In this proposal, we focus on producing the vaccine quickly with adherence to strict quality control, even if greater capital expenditures are required. We thus incorporate larger-scale systems into our proposal to enable production in one batch rather than in multiple smaller batches to ensure that the desired amount of doses reaches consumers as quickly as possible. For instance, the downstream process is anticipated to take 3 days rather than time-intensive alternatives with drastic capital expenditure reductions. Furthermore, we prepare multiple lines in the manual handling stages of the manufacturing process to prevent delays due to line quality.

# 6. Critical-To-Quality Variables

**N/A**

# 7. Product Concepts

**N/A**

# 8. Superior Product Concepts

**N/A**

## 9. Competitive Patent Analysis

The three main types of vaccines currently in development are the subunit vaccine, the viral vector vaccine, and the mRNA vaccine. The recombinant protein, a subunit vaccine, is the most conventional type where antigens on the viral surface are used to trigger immune responses. Viral vector vaccines use genetically engineered capsids of other viruses to deliver antigenic genes. The mRNA vaccine is a relatively recent technology that uses lipid nanoparticles (LNPs) to encapsulate mRNA containing the genetic information for the antigen.

In the manufacturing process of recombinant protein vaccines, insect cells or mammalian cells are first transfected with plasmid containing the antigen. Transfected cells are next grown in a cell culture seed train for mass production. After the cells have grown to the desired amount in the harvest bioreactor, they are infected with viral stock to induce expression of protein. Then, the protein of interest is separated and purified to formulate the vaccine. Published in 2007, an international patent [15] is owned by Merck for its influenza recombinant subunit vaccine. The invention of influenza recombinant subunit vaccine involves the usage of insect cell expression system and saponin adjuvant, which validated our manufacturing model.

For the manufacturing process of mRNA vaccines, many copies of the mRNA of interest are first produced *in vitro* through transcription of DNA in a reactor. Then, the encapsulation of mRNA copies in LNP components involves microfluidic technology to undergo self-assembly. To formulate the vaccine, the LNPs encapsulating the mRNA are isolated and purified. Pfizer and Moderna are companies that have developed the production of LNPs to encapsulate mRNA for the SARS-CoV-2 virus. Published in 2020, a US patent [16] is owned by Moderna for its betacoronavirus mRNA vaccine.

The manufacturing process of viral vector vaccines is similar to that of recombinant protein vaccines in that cells are grown in bioreactors, but different in that cells are infected with viral vectors for propagation. The viral vectors are separated and purified afterward to formulate the vaccine. Published in 2011, an international patent [17] is owned by Janssen for its method of producing Ad26 adenoviral vectors. The method for large-scale production of Ad26 adenoviral vectors involves perfusion systems that grow cells to very high density before infection and propagation of adenoviral vectors after infection. Janssen's patent was used as a reference to apply the perfusion system to our second-to-last bioreactor.

# 10. Preliminary Process Synthesis

## 10.1 Types of Vaccines

### *10.1.1 mRNA*

Messenger RNA, or mRNA, vaccines deliver the genetic information encoding the spike protein [18]. Gene constructs can be quickly transcribed *in vitro* using just the genetic sequence, making production a purely chemical process that does not require cellular growth [19]. The manufacturing process is scalable, which allows for rapid production of vaccines [20]. However, the Pfizer/BioNTech and Moderna vaccines currently in distribution require extreme frozen storage to account for RNA instability, which poses difficulties in the storage and transportation of dosages [21]. Challenges in large-scale production and long-term storage do not have past precedent, thus issues that arise will pose significant costs [20][21]. While mRNA technology is promising, no mRNA vaccines have been licensed in the past for human use and not much data is widely available. Therefore, we did not choose mRNA vaccine platform for our project [18].

### *10.1.2 Viral Vectors*

An alternative method is the viral vector vaccine. In this approach, the genetic sequence of a virus is modified such that it is not pathogenic to the host while still stimulating a strong immunogenic response [22]. The virus is designed in such a way that its pathogenicity is attenuated but still capable of generating antigenic material to which the host reacts, producing a lasting immunogenic response [23]. Unlike traditional attenuated vaccines, the actual viral vector need not be the actual virus itself, but is often a chimeric version of a less potent virus with the particular genetic sequence of the virus of interest [22]. Additionally, to further limit pathogenicity, specific replication-defective vectors have been engineered by precise genetic

editing of the chimeric viruses [24]. However, these replication-deficient vectors have weakened immunogenicity [25]. Additionally, there is a fair amount of concern regarding the ability of viral vector vaccines to combine with wild-type viruses and mutate, thereby regaining full scale pathogenicity [23]. Lastly, very few viral vector vaccines have been approved anywhere in the world, limiting the ability of our senior design group to properly assess such a process [23]. Due to these concerns, the viral vector vaccine's advantages in immunogenicity are outweighed by safety concerns and the lack of prior experience.

### *10.1.3 Subunit*

Current subunit vaccines include recombinant protein, receptor-binding domain (RBD), and virus-like particles (VLPs). We chose to use a recombinant protein-based vaccine using the spike protein as the antigen. The spike protein was the most potent antigen for SARS-CoV and MERS-CoV vaccines [26], and spike protein-based vaccines are known to develop high-titer spike protein-specific antibodies with neutralizing activity [27]. Novavax, the current leader in making recombinant protein-based vaccines for SARS-CoV-2, uses the spike protein as the antigen. The RBD of the spike protein was not chosen as the antigen since it lacks other neutralizing epitopes that are on the full length spike, and adverse events have occurred for some RBD-based vaccines in clinical trials [28]. VLPs were not chosen as the antigen since enveloped baculoviruses and VLPs share similar chemical and physical characteristics, which poses a major drawback in the purification step [29].

## 10.2 Protein-Based Vaccine

### *10.2.1 Spike Protein*

The recombinant protein-based vaccine is a highly conventional vaccine platform, which has been studied for a long time [30]. Spike protein vaccine technology has proven to be safe for immunocompromised people, as the manufacturing of the vaccine does not involve working with live pathogens or genetic material [31]. Recombinant protein-based vaccines do not require frozen storage conditions, which is another major advantage. Novavax is currently developing its recombinant protein-based vaccine with required storage conditions between 2˚C to 8˚C [32], which is much more economical than the frozen storage requirements of mRNA vaccines. However, recombinant protein-based vaccines require the usage of an adjuvant, which is an agent mixed with the vaccine to promote effective immune response. The most common side effects of adjuvants are known to be malaise, fatigue, and headache [21]. Despite these considerations, for our senior design project, we have decided to use the recombinant protein-based vaccine as our vaccine platform.

### *10.2.2 Baculovirus Expression Vector System*

The Baculovirus Expression Vector System (BEVS) will be used to develop the recombinant protein. CERVARIX manufactured by GlaxoSmithKline (GSK) was the first human recombinant protein vaccine produced using the BEVS system [31]. In the BEVS system, insect cells are infected using a recombinant baculovirus with the desired gene. This gene of interest can be made synthetically by obtaining the sequence from a public database, or it can be transcribed from an inactivated virus obtained from the CDC and amplified by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) [33]. As shown in *Figure 10.2*, the gene is

then inserted into the baculovirus plasmid DNA to make the bacmid, which is amplified in a bacterial culture to increase the number of bacmid. The bacteria are lysed to harvest and purify the intracellular bacmid, which is then inserted into adherent insect cells using a transfection reagent. The cells with the bacmid are grown in medium and produce the baculovirus, which are secreted into the supernatant. Cellular debris is removed by ultracentrifugation, and genomic DNA and RNA is digested with nuclease, leaving behind only the baculovirus in the supernatant to be harvested and stored [34].

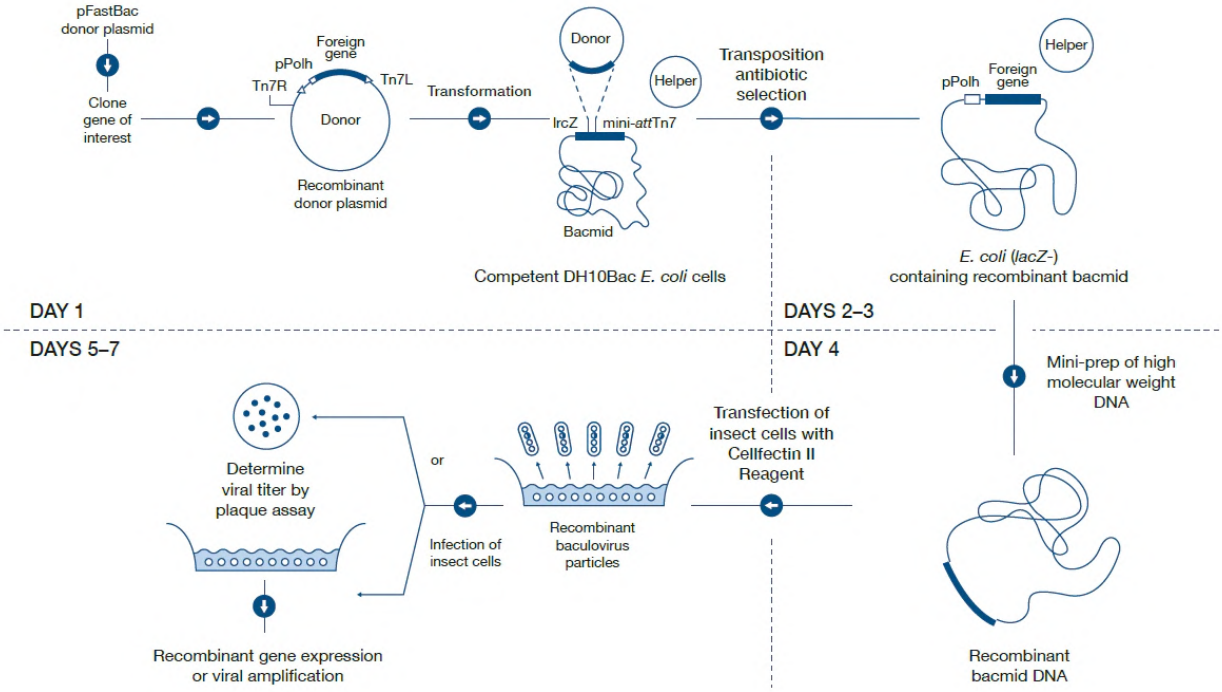


*Figure 10.2.* The Baculovirus Expression Vector System. The gene of interest is inserted into the baculovirus genome to create the recombinant bacmid. The bacmid is transfected into insect cells, which produce and secrete the recombinant baculovirus into the supernatant. (ThermoFisher Scientific)

Various advantages are associated with the BEVS system. High protein yields, large scalability, and fast production are notable benefits [35]. Additionally, there is inherent safety, as the virus only infects invertebrates [35]. Using insect cells is also ideal because they are cheaper than CHO cells yet still perform proper post-translational modification. Although bacteria and yeast cells yield high protein expression at a low cost, they were not considered as viable options

because of their inability to properly perform post-translational modifications. There are some disadvantages to the BEVS system, including quick cell lysis, at 72-96 hours post infection [36]. This can be combated by deleting the gene that produces the apoptotic protein, as well as via various other methods. The required media can also be expensive, but is still cheaper than those for CHO cells. Overall, the BEVS system is optimal, so our group has decided to utilize BEVS to develop a safe and cost-effective vaccine.

### *10.2.3 Cell Line*

The insect cell-baculovirus expression vector system (IC-BEVS) can be manufactured efficiently and scaled up for large-scale production of recombinant proteins. The process consists of cell growth, cell infection with the recombinant baculovirus vector, and purification and recovery of the recombinant protein. The *Spodoptera frugiperda* (*Sf*) cell line is an industry standard cultivated in suspension using serum-free media and is easily detached from cultivation surfaces with gentle agitation, eliminating the need for trypsinization [37]. Our team chose Sf-9 cells because they have higher baculovirus amplification rates, have faster growth rates, and are less fragile — specifically more tolerant to osmotic, pH, and shear stress conditions — than Sf-21 cells [37].

### *10.2.4 Medium*

Sf-9 cells require specific media conditions to grow optimally, thus a variety of media lines from different companies were assessed. Several considerations were examined: maximum cell density, available scale for purchase, GMP capability, and availability of growth condition information. The Thermo Fisher ExpiSf™ system has the largest maximum cell density at

$20\times10^6$ cells/mL using a specialized line of Sf-9 cells [38]. Other versions from Thermo Fisher, such as the Sf-900™ II SFM and Sf-900™ III SFM, have cell densities that were also decent (~$10\times10^6$ cells/mL). Beyond Thermo Fisher, Expression Systems have ESF 921™ media with purported maximum cell densities of $15\times10^6$ cells/mL [39][40][41]. However, certain bioprocessing companies, such as Oxford Expressions Technologies, warn that while cell densities greater than $10^7$ are certainly possible to achieve, they are also harder to sustain for growth cycles [42]. A study of Thermo Fisher's ExpiSf™ line even found that optimal cell density was actually closer to $5\times10^6$ cells/mL [43]. This indicates that maximum cell density above $10^6$ is less significant than cell density below that threshold. Regarding the available scale for purchase, only ExpiSf™ was not sold at industrial scale, whereas other media types had custom orders at sizes up to ten thousands of liters (see brochure from Thermo Fisher Scientific on the Sf-900™ III SFM [40]). Most media appeared to meet the GMP standards, one from Oxford Expressions Technology was not produced at GMP levels, disqualifying this media from our selection [44]. The choice ultimately came down to Sf-900™ II, Sf-900™ III SFM, or ESF 921™. Due to the wider availability of research papers on Sf-900™ II SFM, this media was chosen to ensure our process modeling would be as accurate as possible.

### *10.2.5 Adjuvant*

Since proteins do not induce an adequate immune response on their own, a saponin-based adjuvant is necessary to enhance the response for a protein-based vaccine. While the addition of an adjuvant is outside the scope of our project, adjuvants are necessary for the final product. Saponins produce high antibody titers and better responses for T-independent antigens and CD8+ T cells [45]. They also promote both humoral and cellular immunity, both of which are required

to control viral infections, making saponins ideal for use in subunit vaccines [46]. The Matrix M1™ saponin-based adjuvant in the Novavax SARS-CoV-2 recombinant protein vaccine induced neutralizing antibodies to both SARS and MERS spike proteins more effectively than alum adjuvant [47]. ISCOM™ and ISCOMATRIX™ saponin-based adjuvants were more immunogenic than liposome and protein micelle systems [46]. Saponins were safe and potent in intranasal influenza HA vaccines [46]. They also have a wide range of biological properties, such as anti-inflammatory and antimicrobial activity [48]. However, *Quillaja* saponins have shown haemolytic activity [48], toxicity, and aqueous instability, which are properties that have limited their usage as vaccine adjuvants [46]. In light of these issues, other plant-derived saponin candidates have been investigated with promising results [46][49]. Additionally, while the use of adjuvants elicits side effects, saponins only cause minor local reactions, namely mild to moderate temporary pain, tenderness, and induration at the site of injection [46]. The advantages and potential of saponin adjuvants far outweigh their disadvantages and the benefits of other adjuvant alternatives.

## 10.3 Bioreactors

When assessing the choices of bioreactors available, we evaluated the options along the size variability, sanitation quality, and information provided about the bioreactor. Out of the many bioreactor models, the regular HyPerforma series was chosen for our project. DynaDrive is an alternative bioreactor model that we have considered. The DynaDrive model comes in sizes of 50 L, 500 L, 3000 L, and 5000 L. Because this model has blades that are more robust, it can

support cells with much higher oxygen uptake rate through more intense agitation. However, since DynaDrive is relatively new, many CMO companies would not have this equipment.

### *10.3.1 Sf-9 Cell Seed Train Design*

Our Sf-9 cell seed train consists of 6 stages: (4x) 125 mL flasks, (4x) 500 mL flasks, (3x) 5 L flasks, (3x) 200 L WAVE bioreactors, (1x) 500 L perfusion bioreactor, and (1x) 2000 L batch bioreactor. Quadruplicates are formulated for the 125 mL and 500 mL flasks: one of them is used for baculovirus amplification, and the other three are prepared in consideration of possible damage or contamination to a sample during manual inoculation in between stages. Triplicates are not needed for the 500 L perfusion bioreactor and 2000 L production bioreactor, since the content is transferred through sterile tube welders that prevent the occurrence of contamination. The working volume is scaled up by 10-fold to reduce the number of stages required, thereby reducing the capital cost of equipment. The scale up from the 500 L perfusion bioreactor to the 2000 L batch bioreactor is only 4-fold, since the 2000 L bioreactor is large enough to produce sufficient amount of protein.

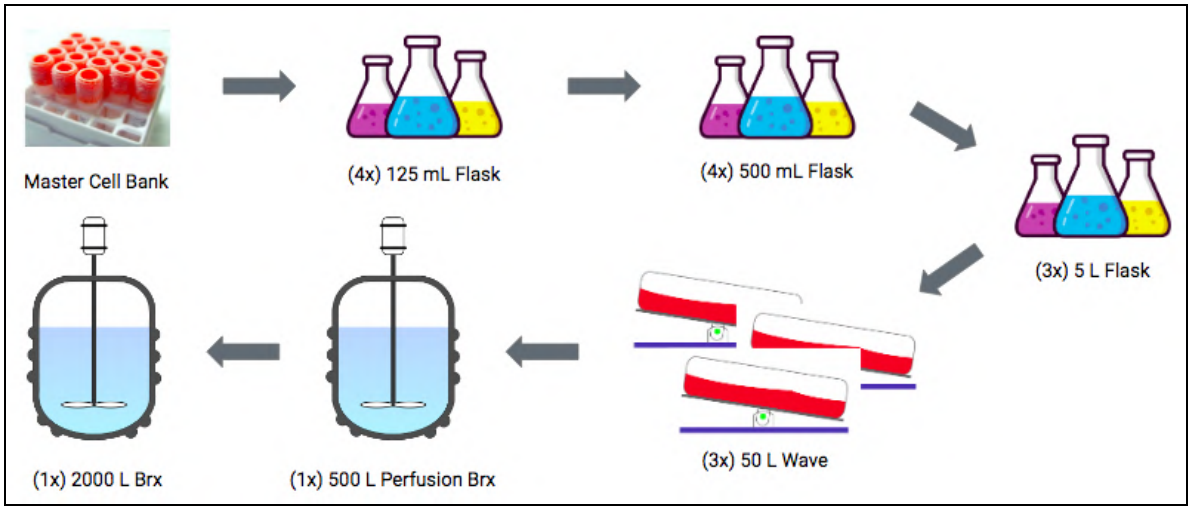


*Figure 10.3.1.* Overview of the Sf-9 Seed Train Design. Sf-9 cells are grown from a master cell bank and scaled up from flasks to bioreactors.

Approximately 4 million cells are grown to around 16 trillion cells to produce 3.75 kg of spike protein, which accounts for the 30% yield loss in the downstream process. Media is pre-filled up to the maximum working volume minus the inoculum volume for all stages except the 125 mL flasks with a 50 mL working volume and the 200 L WAVE bioreactors with a 50 L working volume. The headspace in the bioreactors allows for the addition of materials such as glucose, base, and antifoam as well as the transfer of oxygen and carbon dioxide at the liquid-gas interface. The total amount of media required for the growth stages is 2800 L, and the total amount of media required for the production stage is 1500 L. The extra volume of media required for the 500 L perfusion bioreactor was calculated by using the ratio between the number of cells to the amount of media in the previous stage.

During the growth and production stages, the cells consume oxygen and produce carbon dioxide, which could then react with water to produce carbonic acid and drop the pH level. To maintain the optimum pH of 6.3, sodium hydroxide is added as base if the pH becomes too low. Oxygen, glucose, and glutamine are added to provide nutrients for the cells, air is added to strip carbon dioxide, and antifoam is added to prevent foaming. The time required for the growth stages is around 19 days, and the time required for the production stage is 5 days, totaling an estimate of 24 days for the upstream process.

### *10.3.2 Multiplicity of Infection*

Multiplicity of Infection (MOI) refers to the number of virions added per cell during the infection stage. An MOI value greater than 1 is considered high while an MOI value smaller than 1 is considered low. For our project, we will use a MOI value of 0.01, which is on the lower end. A low MOI value has several advantages in that smaller volumes of virus stock can be purchased

and fewer virus amplification steps are required [50], reducing the operating costs significantly. However, using a low MOI value leads to a two-stage infection process where not all cells are infected immediately after virus addition. Thus, we assumed that 5 days of infection phase would be sufficient for one virion to infect 100 cells, as suggested by our project author Dr. Cohen.

### *10.3.3 Baculovirus Preparation*

Live baculovirus preserved in liquid will be purchased from Applied Biological Materials Incorporated in 10 mL quantities with densities of $10^6$ infectious units/mL [51]. Our baculovirus preparation consists of 3 stages: (2x) 250 mL flasks, (2x) 20 L WAVE bioreactors, and the (1x) 2000 L batch bioreactor used in the Sf-9 seed train detailed above. Duplicates are formulated for the 250 mL flasks and 20 L WAVE bioreactors to account for possible damage or contamination to a sample during manual inoculation in between stages. The 2000 L batch bioreactor does not require duplicates, since all material transfer occurs through sterile tube welders that prevent contamination. The working volume is scaled up 100 fold to reduce the number of stages required, thereby reducing the capital cost of equipment.

Baculovirus preparation starts with a duplicate of two 10 mL virus inoculums, as shown in *Figure 10.3.3*. The virus will first be grown in the 250 mL flasks and subsequently propagated through viral infection in the 20 L WAVE bioreactors before transfer to the production bioreactor. As previously mentioned, we will use an MOI value of 0.01 at each stage to scale up from approximately 20 million viral infectious units to 200 billion infectious units for the 2000 L batch bioreactor to produce the required 3.75 kg of protein. A working volume of 200 mL (including inoculum) will be used for the 250 mL shake flasks, and a working volume of 20 L

will be used for the WAVE bioreactors. For information on the 2000 L bioreactor, refer to the Sf-9 cell seed train (Section 10.3.1) above.

During the infection of cells with baculovirus, cell growth is not likely to occur, as the virus will take over cellular machinery for replication and prevent cell growth. The WAVE bioreactors will require air to provide oxygen delivery and carbon dioxide stripping for the cells. No fresh media is required for the baculovirus amplification, since media will all come from the Sf-9 cell seed train. Total time required for baculovirus amplification, before the 2000 L bioreactor stage, is about 11 days.

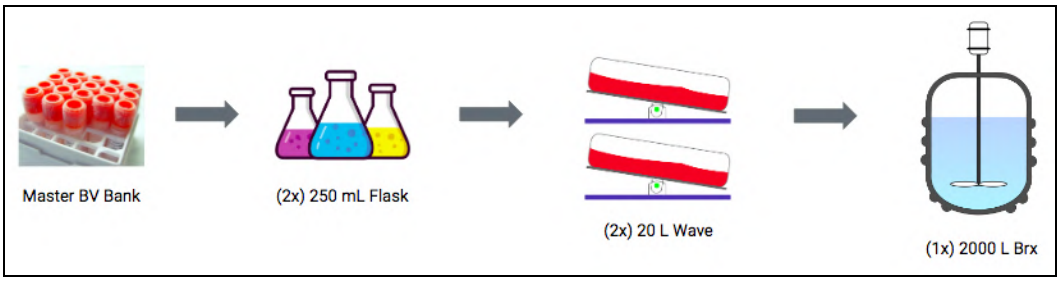


*Figure 10.3.3.* Overview of the Baculovirus Amplification Process. Recombinant baculovirus is amplified from a master bank and scaled up from flasks to bioreactors.

## 10.4 Membrane Filtration

Membrane processes are filtration systems that are scaled by stacking multiple cartridges in parallel. They are adaptable to disposable formats, which eliminate the risk of contamination and the need for cleaning validation between runs. Recovery yields are frequently above 90% [52]. The filters are asymmetric, meaning larger pores are on the entry side and smaller pores are on the exit side for efficient filtration [53]. Depth filtration and tangential flow diafiltration are employed in the clarification, purification, and polishing processes. Dead-end disposable depth filters efficiently retain larger cell debris, bacteria, and some viral particles, allowing for the smaller spike protein to pass through the membranes [53]. Diafiltration separates the spike

protein in the retentate from smaller solutes, such as salts and sugars in the permeate. Fresh diafiltration buffer is continuously added at the same rate of permeate removal, thus the tank volume remains constant throughout the process. Diafiltration is run in a tangential flow configuration to minimize plugging for more efficient filtration. *Equation {1}* was used to determine the membrane area required, assuming no salt is rejected by the membrane [54].

$$ln\frac{c_s}{c_{s0}} = -\frac{Qt}{V} \qquad \{1\}$$

where
$V$ = volume of solution being desalted (L)
$c_s$ = concentration of salt in volume V (g/L)
$c_{s0}$ = initial concentration of salt (g/L)
$Q$ = filtration rate (L/hr)
$t$ = time required for diafiltration (hr)

## 10.5 Chromatography Columns

Chromatography columns are packed with specialized resins to selectively isolate the protein of interest. In order to design the chromatography columns, the type of resin-protein interactions, the specific resin to be used, the amount of resin, the sizing of the column, the buffer volumes, the flow rates, and the process times were selected or calculated. An affinity chromatography column plus an ion-exchange chromatography column was the most commonly used combination in literature [55]. Use of a gel filtration (size-exclusion chromatography) column was rejected because of the additional yield losses it would cause [55].

For the affinity chromatography column, immobilized metal-affinity chromatography (IMAC), otherwise known as his-tag chromatography, appeared to be relatively inexpensive for affinity resins that typically experience high costs, and the histidine residue seemed well adapted for insertion into a recombinant protein [56]. For IMAC resins, there are two broad categories:

cobalt-based resins and nickel-based resins. The cobalt resins have higher binding specificity, but the nickel resins have higher binding capacity, meaning more protein binds to the resin [57]. Because purity is prioritized in pharmaceutical manufacturing, cobalt-based resins are a better choice for IMAC resins. The Cobalt Superflow Agarose resin, specifically, was selected because of its high binding capacity and optimal flow rate.

The two categories of ion-exchange chromatography resins are cation exchange resins and anion exchange resins. From research, anion-exchange resins had superior binding capacity and higher percent recovery than cation-exchange resin for certain products, so anion-exchange resins were chosen [58]. The Poros XQ resin, in particular, was selected because of its high binding capacity and endurance across pH ranges.

Having determined the type of resins, the dynamic binding capacity was used to estimate the total amount of resin needed. The approximate amount of protein that needed to be bound at each stage was calculated using percent recovery yields of downstream units from literature sources. The total amount of resin required for the chromatography columns was then calculated using *Equation {2}*.

$$\frac{P}{DBC} = R \qquad \{2\}$$

where $P$ = total amount of protein needed to be bound (mg)
$DBC$ = dynamic binding capacity (mg/mL)
$R$ = amount of resin required (mL)

*DBC* specifies the mass of protein that can be bound to a volumetric quantity of resin. Based upon the amount of resin required, the number of reuses available per resin, and the economics of the system, conducting 5 runs per cycle for the IMAC resins and just 1 run for the ion-exchange resins was reasonable. To calculate the total amount of resin required, the number of cycles were divided using *Equation {3}*.

$$\frac{R}{N} = R' \quad \{3\}$$

where $R$ = amount of total resin required (mL)
$N$ = number of cycles
$R'$ = volumetric amount of resin required per cycle (mL)

Next, the chromatography columns were sized. Based on the advice of consultant Dr. Stijn Koshari, the packed-bed heights were kept around 250 cm, the diameters were calculated to fit *R'*, and then the nearest sized columns from Millipore and Cytiva were identified. By recycling resin over multiple cycles, both resin amount and column size could be cut down.

Buffer volumes for chromatography columns are predominantly dependent on column volume, typically from 5 to 10 column volumes of buffer. Once column volumes were obtained, the required amounts of buffer for the two chromatography processes were calculated.

With column volumes determined, process flow times for the chromatography columns could also be calculated. Using the linear flow rate and column diameter provided by the vendor, the volumetric flow rate could be calculated.

For the IMAC resins, the recommended linear flow of 150 cm/hr was used for stages that include recombinant protein, which is squarely in the recommended operational range. For stages that exclude the protein, a doubled flow rate of 300 cm/hr was used, which is at the maximal recommended operation range.

For the ion exchange resins, a recommended linear flow rate of 300 cm/hr was used. The pressure drop at this linear flow rate is less than 3 bar, which keeps the operation within the safe operational range.

Using the volumetric flow rates described above, the necessary process time for the two chromatography stages was calculated with *Equations {4}* and *{5}*. Again, the buffer volume required was column volume dependent, whereas the amount of protein extract flowing through

was resin dependent. For the IMAC chromatography, where the resin was used over 5 cycles, the protein extract was brought over in installments of ⅕ at a time.

$$\text{Cycle Process Time (Per run): } t = \Sigma \frac{v_{buffer}}{v_{buffer}'} + \frac{v_{\frac{protein\ extract}{N}}}{v_{protein\ extract}'} \quad \{4\}$$

$$\text{Total Process Time: } t = N * \Sigma \frac{v_{buffer}}{v_{buffer}'} + \frac{v_{protein\ extract}}{v_{protein\ extract}'} \quad \{5\}$$

where
$v_x$ = volume of buffer or protein extract (L)
$v_x'$ = volumetric flow rate of buffer or protein extract (L/hr)
$t$ = time to flow through
$N$ = number of cycles

There is one key difference in calculations between the IMAC and ion-exchange resins. For the IMAC resins, where multiple cycles occurred, we accounted for the per-cycle cleaning time because the column had to be regenerated each cycle. For the ion-exchange resins, where only one run is required, the cleaning time could be ignored, since cleaning could be done later in parallel with other parts of the downstream process.

## 10.6 Downstream Process Alternatives

### *10.6.1 Cell Lysis*

Cell lysis is a process that involves breaking open the insect cells to obtain intracellular protein content for further purification. There are many cell lysis techniques, including both chemical and physical mechanisms. However, the baculovirus has genes (chitinase and v-cathepsin) that encode for proteins involved in early cell lysis and death [36]. Specifically, the cells will lyse 3 to 4 days post-infection. For many situations, early lysis is undesirable and its genome is altered in order to delay lysis [36]. However, based on the designed seed train, cell lysis would have occurred by the 5th day post-infection. Therefore, cell lysis is not a necessary

step of our downstream process. We assume that 100% of the cells will be lysed by the time we begin our downstream process and protein purification.

### *10.6.2 Centrifugation*

Historically, the first phase of downstream processes included Liquid-Liquid Extraction (LLE), centrifugation and microfiltration, but membrane-filtration technologies are far more used nowadays [59]. Ultracentrifugation has been commonly used in the past, but it is highly inefficient in that combination of ultracentrifugation, and size exclusion chromatography can only obtain up to 75% recovery [60], which is far below desired percent recovery for a clinical grade product [59]. Also, ultracentrifugation is time-consuming and labor intensive , with poor reproducibility and limited scalability [59][60].

Centrifugation is recommended as the first step in the downstream process only if cell density is high. High cell density generally refers to VCD greater than 10 million cells / mL, but the VCD for our harvest batch is 8 million cells / mL. Since our cell density is low, centrifuging to get rid of the cell debris pellet is inefficient. Therefore, centrifugation was not implemented in our downstream process design.

## 10.7 Plant Locations

China, India, South Korea, and the United States were considered for manufacturing before deciding on the United States. Consultation with Dr. Alex Marchut provided the following insights. First, companies have become more wary of arranging manufacturing in China, as its government has swiftly changed policies in the past that have caused many CMOs to shut down.

India and South Korea have more lenient governments, thus companies are more open to working with CMOs in these countries. Finally, reshoring in the United States has become more popular, especially during the pandemic, and trends have shown manufacturing shifting to the Southeastern region.

None of the required manufacturing equipment, materials, utilities, or labor requirements are geographically restricting. Thus, the location of the manufacturing facility was completely dependent on the location of the plant(s) operated by the CMO approached. Dr. Cohen noted that BioGen is a favorite choice for CMO, which heavily motivated our selection of the company. BioGen has two facilities dedicated to pharmaceutical operations and technology: one in North Carolina and the other in Switzerland. The plant in North Carolina utilizes single-use technology that replaces the traditional stainless steel systems, which aligns with much of our upstream and downstream processes [61]. Single-use technology eliminates contamination risk and cleaning validation required by traditional stainless steel equipment, and it also allows for more process flexibility, which has made it such a popular choice in the biopharmaceutical industry. Additionally, the plant is located in the Southeastern region of the US, as recommended by Dr. Marchut. These factors made the BioGen facility in North Carolina a suitable choice as the plant location.

# 11. Assembly of Database

## 11.1 Cell Bank

A cell bank containing 1 mL of cell stock in 2 mL vials is at a temperature of -80˚C [62]. Glycerol or DMSO is included in the cell stock as antifreeze to protect cells from low temperatures. An important precaution is that the population of cells in each cell bank vial has some amount of uncertainty and variability. Cell bank inoculum with 50 million cells per vial is available for purchase online.

## 11.2 Cell Growth Conditions

### *11.2.1 pH*

The optimum pH for Sf-9 cells in both growth and infection phases is between 6.2 and 6.4 [63]. Therefore, the pH control and deadband for all stages of the upstream process are set at 6.3 and 0.2, respectively.

### *11.2.2 Temperature*

The optimum temperature for Sf-9 cells in terms of both growth rate and viable cell density (VCD) is around 27˚C [64] and 28˚C [66]. Growth rate decreases gradually with temperature, and is significantly lower at 25˚C. VCD decreases at 30˚C [65]. For the production phase, recombinant proteins are similarly expressed at 22˚C, 25˚C, and 27˚C. Although total protein yields are similar in all three temperatures, lower temperature causes cells to get infected at a slower rate. Thus, the temperature control and deadband for growth stages are set at 27.5˚C

and 1.0˚C, respectively, and those for the production stage are set at 26.0˚C and 2.0˚C, respectively.

### *11.2.3 Dissolved Carbon Dioxide*

The optimum dissolved $CO_2$ level for Sf-9 cell growth is between 0 to 37 mmHg $CO_2$ [67]. For our project, the dissolved $CO_2$ level is set at 30 mmHg for the bioreactors in growth and production stages.

### *11.2.4 Nutrients*

The carbon source and nitrogen source used for Sf-cells are glucose and glutamine, respectively. Since prepared media transfer through sterile filters to fill flasks and bioreactors, the nutrients inside prepared media must be fully dissolved. Hence, glutamine is used as the nitrogen source because it is fully soluble in water, compared to the commonly used yeast extract that is not fully soluble in water.

### *11.2.5 Base*

The base used to control the pH level for our bioreactors is 1 M sodium hydroxide. The amount of base used in our project is based on a test by Thermo Fisher Scientific, where 27 mL of 0.1 M sodium hydroxide was used as base for its 5 L HyPerforma® rocking bioreactor [68].

### *11.2.6 Antifoam*

The antifoam used to prevent foaming is in an emulsion form. Since the foam covers the cell surface and may affect cell growth and metabolism, the use of antifoam minimizes the

amount of foam present. However, since antifoam cannot differentiate between cell surface, wall surface, and liquid surface, using too much antifoam can decrease the interfacial surface energy of cells and thereby slow down cell metabolism. Thus, the amount of antifoam used in our project is 0.3% of total volume for each bioreactor [69].

### *11.2.7 Osmolality*

One of the advantages of insect cells over mammalian cells is that insect cells are less sensitive to increase in osmolality than mammalian cells [37]. The osmolality for Sf-9 cells is adjusted between 360 and 380 mOsm/kg using NaCl [65].

### *11.2.8 Shear Stress*

*Agitation Rate*

In order to maintain a growth rate of at least 0.032 $hr^{-1}$, the shear stress should be kept to less than 1 $N/m^2$ [70]. Agitation rate of 250 RPM negatively impacted cell growth due to high shear stress, whereas agitation rates of 50 RPM and 100 RPM did not [63].

*Aeration Rate*

Aeration rate must also be monitored to ensure that the specific growth rate does not decrease. Specifically, data suggests that if agitation rate is operating at 200 RPM, the aeration rate must be kept below 0.04 vvm (volume air per volume liquid per minute) to maintain the maximum specific growth rate [70].

*Bubble Diameter*

Bubble diameter is also crucial to monitor because bubbles can cause cells to rupture. When the bubble diameter is too small, the specific death rate increases. *Figure 11.1* below

depicts the effect of bubble diameter on death rate of Sf-9 cells when sparged at a gas flow rate of 10 mL/min [70]. The optimal bubble diameter should be greater than 0.2 cm, which can be controlled by the sparger.

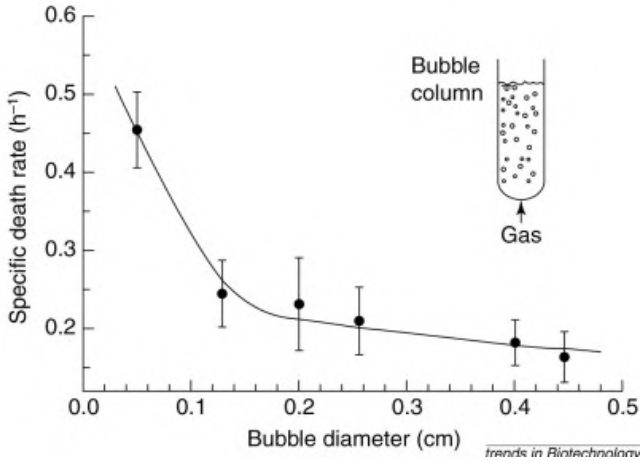


*Figure 11.1*. Specific Sf-9 Cell Death Rate vs. Gas Bubble Diameter [70]. As the bubble diameter of the entering gas increases, the specific death rate of Sf-9 insect cells in the column decreases.

## 11.3 Cell Growth Kinetics

Rhiel et al. [71] cultivated Sf-9 cells in Sf-900™ II SFM at a temperature of 27°C and a pH between 6.30 and 6.32. Sf-9 cells grown in Sf-900™ II SFM, represented by the hollow circles in *Figure 11.2*, had a growth rate of 0.033 $hr^{-1}$ and a doubling time of 21 hr, reaching a maximum VCD of $8.1\times10^6$ cells/mL after growing for 4 days.

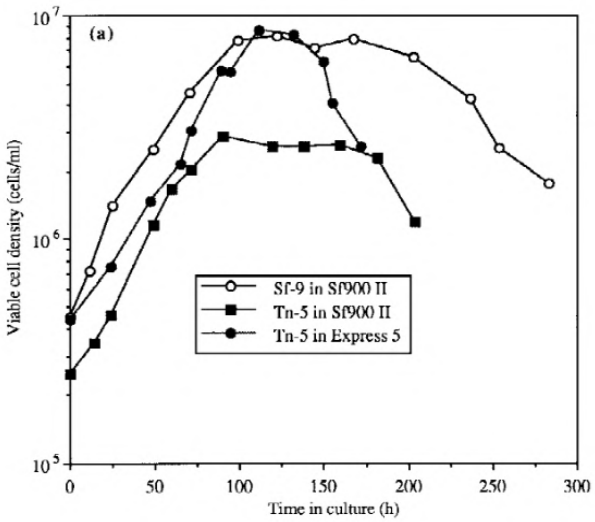


*Figure 11.2*. Viable Insect Cell Density vs. Time in Culture [71]. As insect cells are grown in suspension, the viable cell density increases, peaks, then decreases. The growth kinetics are dependent on the medium used.

## 11.4 Cell Metabolic Rates

### *11.4.1 Oxygen Consumption*

Cells were grown in 65% $O_2$ concentration and were infected at 50% $O_2$ concentration. Studies, overall, seemed to indicate that cells were not so affected by variations in environmental oxygen concentration, as long as the oxygen concentration stayed above 10% and was less than 110% for the growth phase [72]. Optimally, cells are to be grown between 40% and 70% $O_2$, but deleterious effects were not seen when $O_2$ concentration slightly deviated from this range [73]. Studies also indicated that while cell oxygen consumption during the infection phase does increase to as much as 1.3 times the rate of consumption during growth phase [74], the optimal oxygen concentration for the infection phase is, paradoxically, slightly less at around 50% [75]. The specific oxygen uptake rate (sOUR) is around 0.22 to 0.26 μM $O_2/10^6$ cell/hr during the growth phase [63] and 0.2 to 0.76 μM/$10^6$ cell/hr for the infection phase [37]. For the infection

phase, the high end value of sOUR is more reasonable due to the increase in oxygen consumption during infection.

### *11.4.2 Carbon Dioxide Production*

The specific carbon dioxide production rate ($qCO_2$) for uninfected Sf-9 cells is between 2.6 and 7.4 mmol/$10^9$ cells/day, and that for virus-infected Sf-9 cells increases to 10.3 mmol/$10^9$ cells/day [76].

### *11.4.3 Respiratory Quotient*

Respiratory Quotient (RQ) is the ratio of carbon dioxide produced by the cells to oxygen consumed by the cells. The RQ for uninfected Sf-9 cells is 1.07 ± 0.06, and that for virus-infected Sf-9 cells is 1.12 ± 0.03 [76].

### *11.4.4 Metabolic Consumption and Production*

Insect cells consume glucose and glutamine while producing lactate and ammonia as part of their metabolic process. Rhiel et al. [71] cultivated Sf-9 cells in Sf-900™ II SFM at a temperature of 27°C and a pH between 6.30 and 6.32. Under these conditions, the cells consumed $2.4\times10^{-17}$ and $1.7\times10^{-17}$ moles of glucose per cell per second in the growth phase and infection phase, respectively. The cells consumed $1.7\times10^{-17}$ and $0.5\times10^{-17}$ moles of glutamine per cell per second in the growth phase and infection phase, respectively. The lactate and ammonia by-products are produced in negligible amounts.

### *11.4.5 Protein Production*

Sf-9 cells in TNM-FH medium produce on average 230 μg of protein per $10^6$ cells over the course of 5 days [77]. Since data on protein production in Sf-900™ II SFM could not be obtained, we assumed the cells would behave similarly in the media and would thereby produce the same amount of protein per million cells.

## 11.5 Protein Stability Concerns

### *11.5.1 Temperature*

The spike protein must be kept in a certain temperature and pH range to ensure that it does not denature. When the temperature is above 40˚C, the RBD will begin to adapt to a closed conformation [78]. Fortunately, the S1 and S2 domains proved stable at temperature conditions ranging from 10˚C to 50˚C, so there are little temperature stability concerns.

### *11.5.2 pH*

A study on the ability of the spike protein to bind to the ACE2 receptor at different pH levels found that stability was maintained at pH levels above 5.5 [79]. The spike protein will thus be inactive at low pH levels. However, the protein folding is only temporary, and stability is regained when the pH is increased.

# 12. Process Flow Diagram and Material Balance

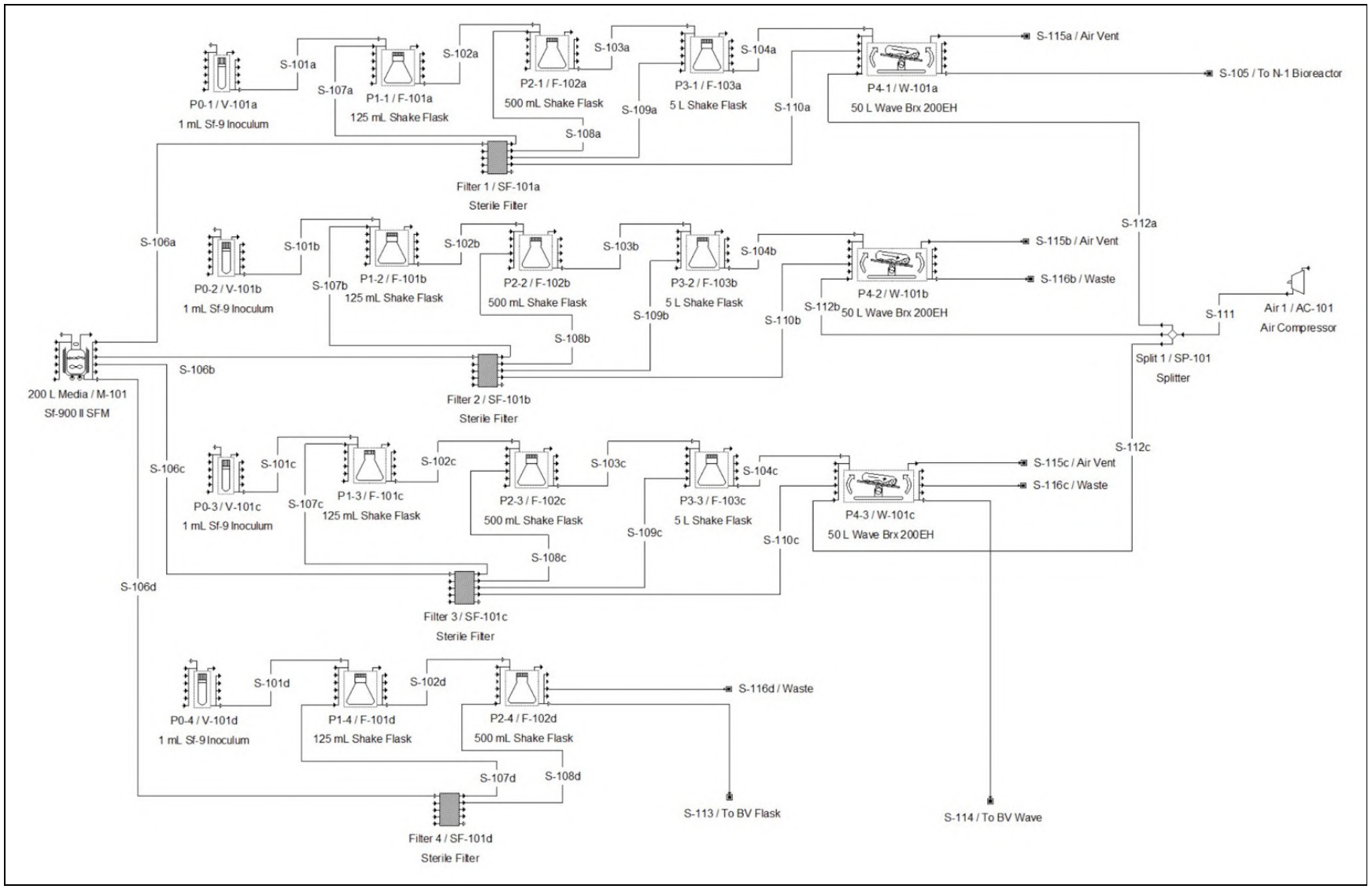


*Figure 12.1.* Process Flow Diagram for the Upstream Process (Section 1)

Table 12.1. Material Balance for Upstream Process (Section 1)

| **Upstream (Section 1)** | | | | | | |
|---|---|---|---|---|---|---|
| | S-101a/b/c/d | S-102a/b/c/d | S-103a/b/c | S-104a/b/c | S-105 | |
| Temperature (˚C) | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 | |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 | 1.21 | |
| Cell Matter (L) | 0.001 | 0.05 | 0.5 | 5 | 50 | |
| | S-106a/b/c | S-106d | S-107a/b/c/d | S-108a/b/c/d | S-109a/b/c | S-110a/b/c |
| Temperature (˚C) | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 |
| Media (L) | 49.999 | 0.499 | 0.049 | 0.45 | 4.5 | 45 |
| | S-111 | S-112a/b/c | S-113 | S-114 | S-115a/b/c | |
| Temperature (˚C) | 25 | 25 | 27.5 | 27.5 | 27.5 | |
| Pressure (bar) | 1.21 | 1.21 | 1.01 | 1.21 | 1.01 | |
| Cell Matter (L) | - | - | 0.36 | 39.6 | - | |
| Air (kg) | 33 | 11 | - | - | 11 | |
| | S-116b | S-116c | S-116d | | | |
| Temperature (˚C) | 27.5 | 27.5 | 27.5 | | | |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | | | |
| Cell Matter (L) | 50 | 10.4 | 0.14 | | | |

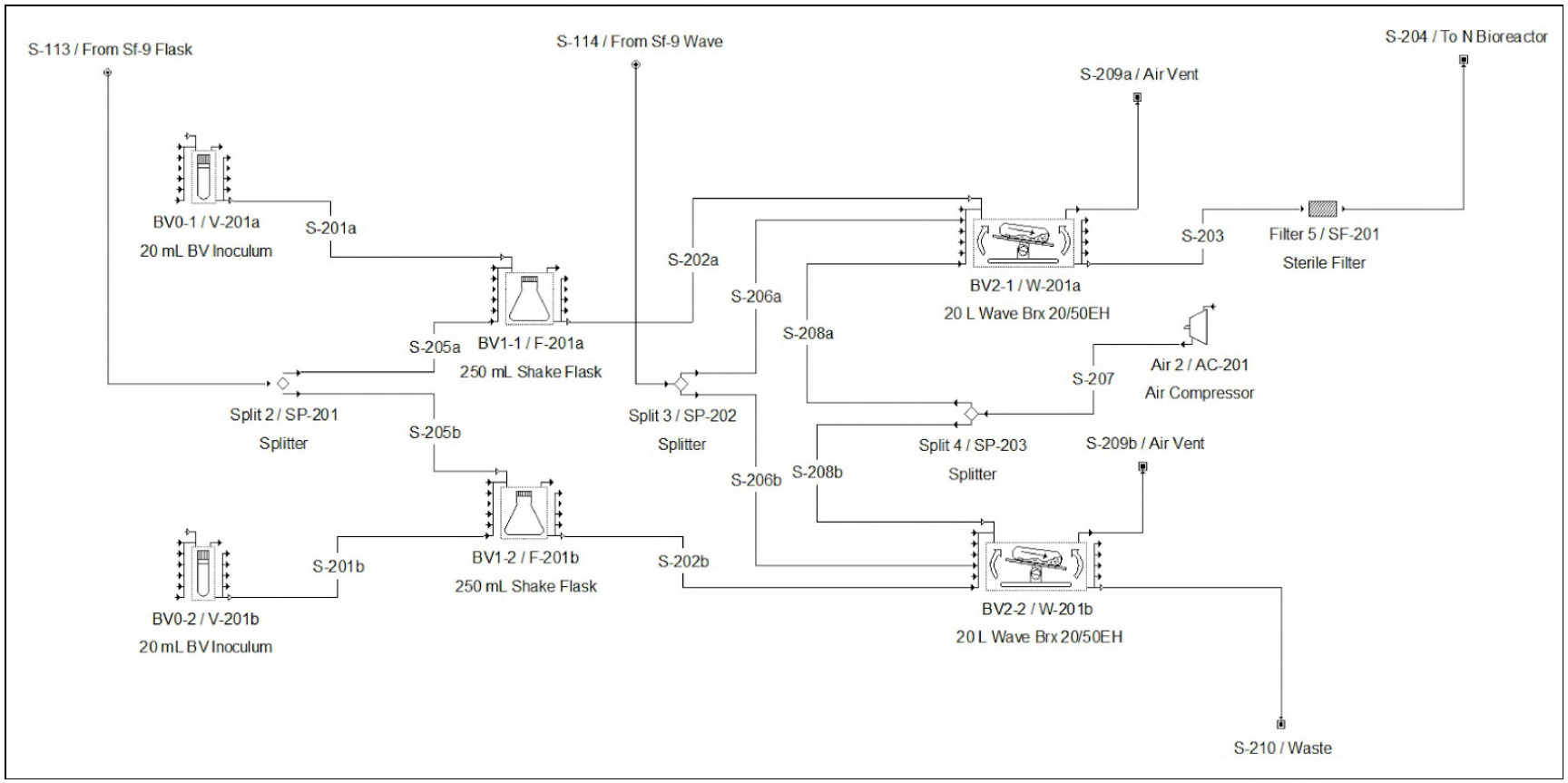


*Figure 12.2.* Process Flow Diagram for the Upstream Process (Baculovirus Preparation)

Table 12.2. Material Balance for Upstream Process (Baculovirus Preparation)

| **Upstream (Baculovirus Preparation)** | | | | | | |
|---|---|---|---|---|---|---|
| | S-113 | S-114 | S-201a/b | S-202a/b | S-203 | S-204 |
| Temperature (˚C) | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 |
| Pressure (bar) | 1.01 | 1.21 | 1.01 | 1.01 | 1.11 | 1.11 |
| Baculovirus Matter (L) | - | - | 0.02 | 0.2 | 20 | 20 |
| Cell Matter (L) | 0.36 | 39.6 | - | - | - | - |
| | S-205a/b | S-206a/b | S-207 | S-208a/b | S-209a/b | S-210 |
| Temperature (˚C) | 27.5 | 27.5 | 25 | 25 | 27.5 | 27.5 |
| Pressure (bar) | 1.01 | 1.21 | 1.11 | 1.11 | 1.01 | 1.01 |
| Baculovirus Matter (L) | - | - | - | - | - | 20 |
| Cell Matter (L) | 0.18 | 19.8 | - | - | - | - |
| Air (kg) | - | - | 4.4 | 2.2 | 2.2 | - |

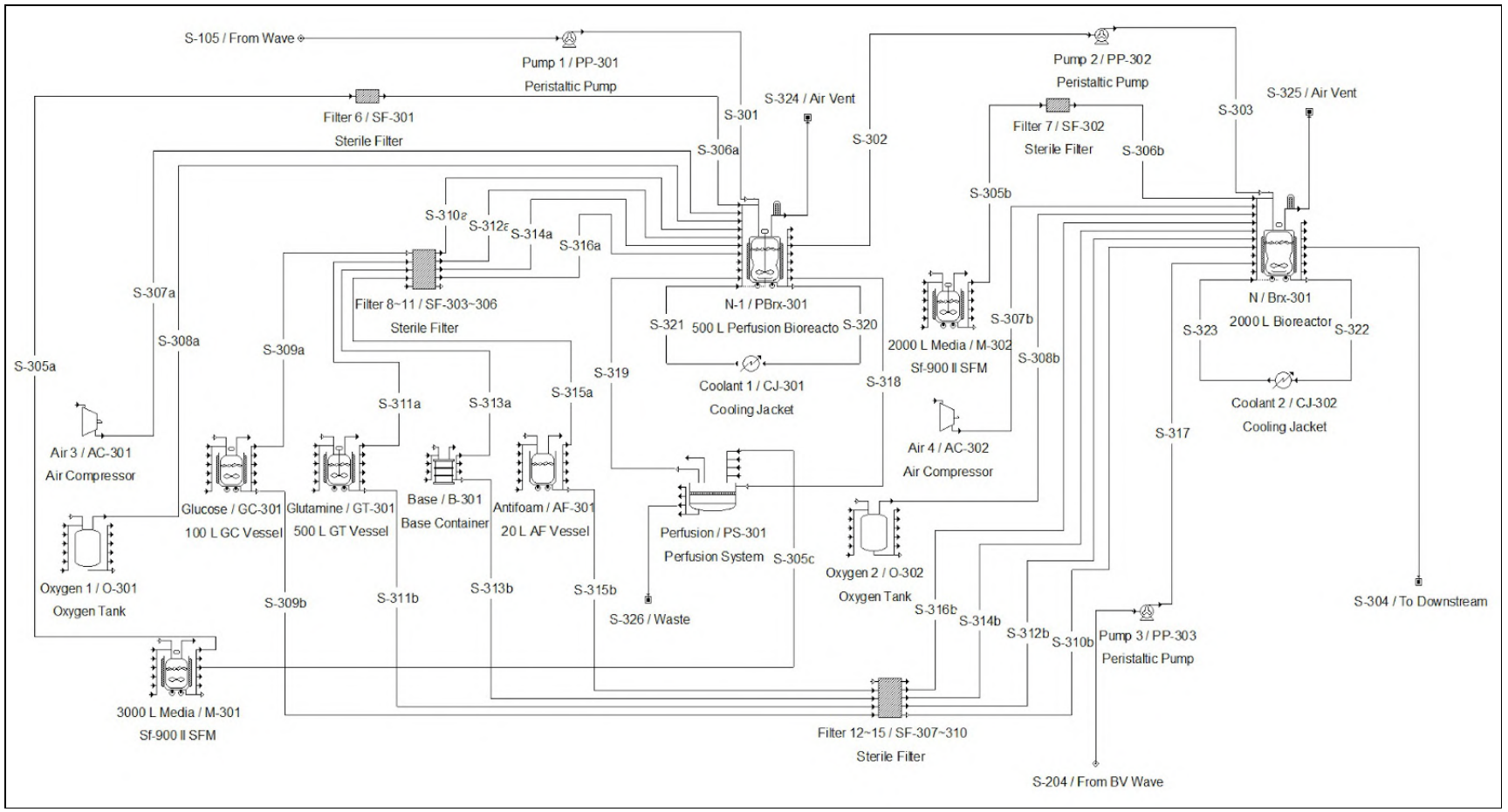


*Figure 12.3.* Process Flow Diagram for the Upstream Process (Section 2)

Table 12.3. Material Balance for Upstream Process (Section 2)

| **Upstream (Section 2)** | | | | | | |
|---|---|---|---|---|---|---|
| | S-105 | S-204 | S-301 | S-302 | S-303 | S-304 |
| Temperature (˚C) | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 | 26 |
| Pressure (bar) | 1.21 | 1.11 | 1.36 | 1.04 | 1.54 | 1.04 |
| Cell Matter (L) | 50 | - | 50 | 500 | 500 | - |
| Baculovirus Matter (L) | - | 20 | - | - | - | - |
| Protein Extract (L) | - | - | - | - | - | 2000 |
| Protein (kg) | - | - | - | - | - | 3.75 |
| | S-305a/306a | S-305b/306b | S-305c | S-307a | S-307b | |
| Temperature (˚C) | 27.5 | 26 | 27.5 | 25 | 25 | |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 2.16 | 2.23 | |
| Media (L) | 450 | 1472.9 | 2190.4 | - | - | |
| Air (kg) | - | - | - | 229.6 | 1377.6 | |
| | S-308a | S-308b | S-309a/310a | S-309b/310b | S-311a/312a | S-311b/312b |
| Temperature (˚C) | 25 | 25 | 27.5 | 26 | 27.5 | 26 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 |
| Oxygen (kg) | 82.4 (50% O2) | 61.8 (100% O2) | - | - | - | - |
| Glucose (kg) | - | - | 30.5 | 21.6 | - | - |
| Glutamine (kg) | - | - | - | - | 11.3 | 5.15 |
| | S-313a/314a | S-313b/314b | S-315a/316a | S-315b/316b | S-317 | |
| Temperature (˚C) | 27.5 | 26 | 27.5 | 26 | 27.5 | |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 | 1.34 | |
| Base (L) | 1.5 | 1.1 | - | - | - | |
| Antifoam (L) | - | - | 8.1 | 6 | - | |
| Baculovirus Matter (L) | - | - | - | - | 20 | |
| | S-318 | S-319 | S-320 | S-321 | S-322 | S-323 |
| Temperature (˚C) | 27.5 | 27.5 | 4.44 | 27.5 | 4.44 | 26 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Pressure (bar) | 1.04 | 1.04 | 1.01 | 1.01 | 1.01 | 1.01 |
| Media (L) | 2200 | 2200 | - | - | - | - |
| Chilled Water (kg) | - | - | 2964.1 | 2964.1 | 3314 | 3314 |

| | S-324 | S-325 | S-326 |
|---|---|---|---|
| Temperature (˚C) | 27.5 | 26 | 27.5 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 |
| Air (kg) | 229.6 | 1377.6 | - |
| Oxygen (kg) | 74.4 (50% O2) | 55.8 (100% O2) | - |
| Carbon Dioxide (kg) | 23.6 | 17.7 | - |
| Media (L) | - | - | 2200 |

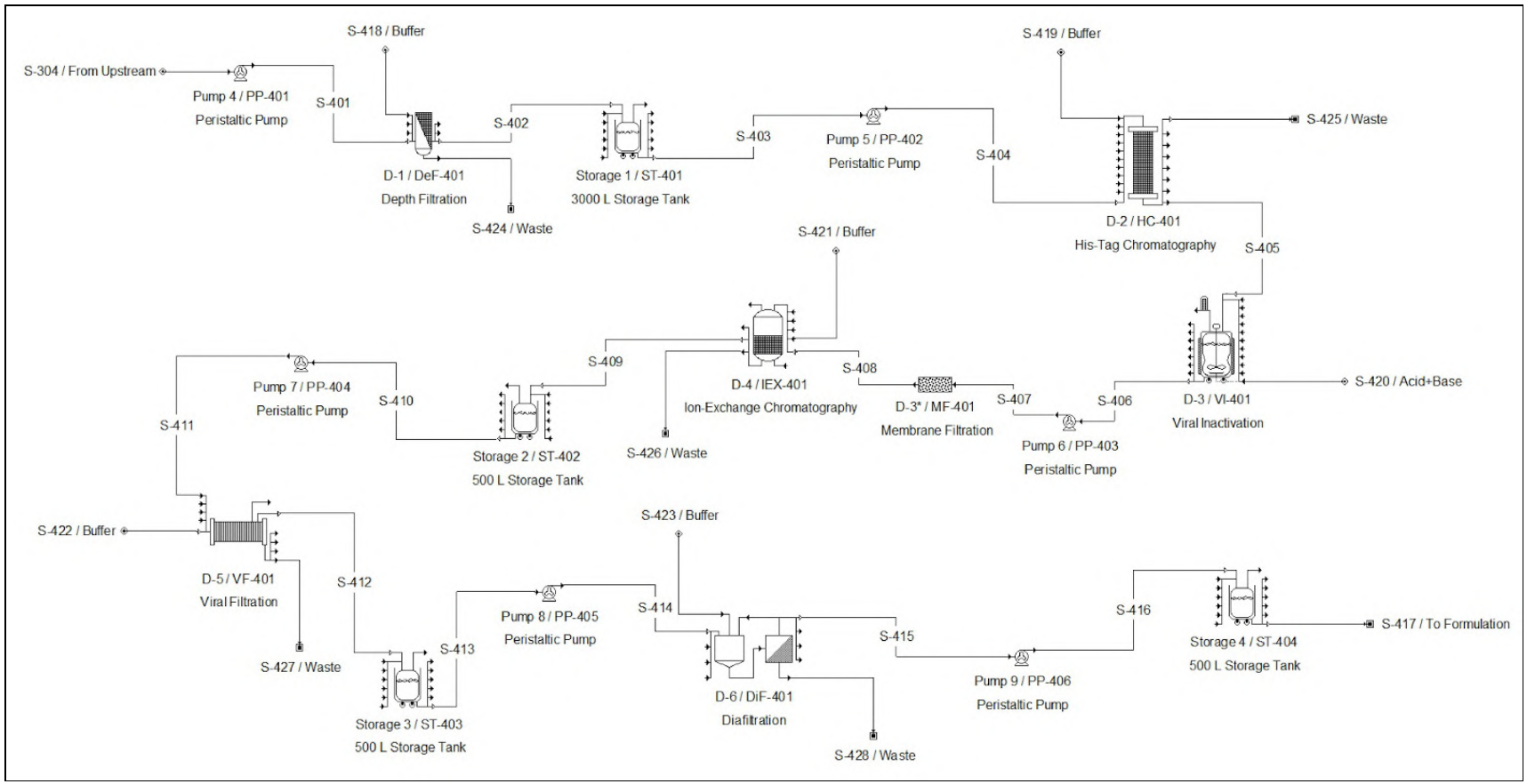


*Figure 12.4*. Process Flow Diagram for the Downstream Process

Table 12.4. Material Balance for Downstream Process

| **Downstream** | | | | | | |
|---|---|---|---|---|---|---|
| | S-304 | S-401 | S-402 | S-403 | S-404 | S-405 |
| Temperature (˚C) | 26 | 26 | 25 | 25 | 25 | 25 |
| Pressure (bar) | 1.04 | 3.14 | 1.04 | 1.04 | 2.03 | 1.04 |
| Protein (kg) | 3.75 | 3.75 | 3.38 | 3.38 | 3.38 | 3.04 |
| Protein Extract (L) | 2000 | 2000 | 2366 | 2366 | 2366 | 1257 |
| | S-406 | S-407 | S-408 | S-409 | S-410 | S-411 |
| Temperature (˚C) | 25 | 25 | 25 | 25 | 25 | 25 |
| Pressure (bar) | 1.04 | 3.44 | 3.44 | 1.04 | 1.04 | 3.14 |
| Protein (kg) | 3.04 | 3.04 | 2.98 | 2.68 | 2.68 | 2.68 |
| Protein Extract (L) | 1353 | 1353 | 1353 | 370.4 | 370.4 | 370.4 |
| | S-412 | S-413 | S-414 | S-415 | S-416 | S-417 |
| Temperature (˚C) | 25 | 25 | 25 | 25 | 25 | 25 |
| Pressure (bar) | 1.04 | 1.04 | 1.28 | 1.01 | 1.09 | 1.01 |
| Protein (kg) | 2.63 | 2.63 | 2.63 | 2.60 | 2.60 | 2.60 |
| Protein Extract (L) | 395.9 | 395.9 | 395.9 | 401.7 | 401.7 | 204.7 |
| | S-418 | S-419 | S-420 | S-421 | S-422 | S-423 |
| Temperature (˚C) | 25 | 25 | 25 | 25 | 25 | 25 |
| Pressure (bar) | 3.11 | 2.99 | 1.04 | 3.41 | 3.11 | 1.25 |
| Depth Filtration Wetting Buffer (L) | 458.5 | - | - | - | - | - |
| Depth Filtration Flushing Buffer (L) | 458.5 | - | - | - | - | - |
| His-Tag Equilibration Buffer (L) | - | 1257 | - | - | - | - |
| His-Tag Wash Buffer (L) | - | 2513 | - | - | - | - |
| His-Tag Elution Buffer (L) | - | 1257 | - | - | - | - |
| His-Tag Regeneration Buffer (L) | - | 1257 | - | - | - | - |
| His-Tag Water Buffer (L) | - | 1257 | - | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Viral Inactivation Acid (L) | - | - | 31.4 | - | - | - |
| Viral Inactivation Base (L) | - | - | 65.3 | - | - | - |
| IEX Equilibration Buffer (L) | - | - | - | 265 | - | - |
| IEX Elution Buffer (L) | - | - | - | 307 | - | - |
| IEX Equilibration Wash Buffer (L) | - | - | - | 159 | - | - |
| IEX NaCl Buffer (L) | - | - | - | 265 | - | |
| IEX Ultrapure Water (L) | - | - | - | 529 | - | - |
| IEX Acetic Acid Wash (L) | - | - | - | 265 | - | - |
| Viral Filtration Wetting Buffer (L) | - | - | - | - | 26 | - |
| Viral Filtration Flushing Buffer (L) | - | - | - | - | 26 | - |
| Diafiltration Base Buffer (L) | - | - | - | - | - | 68 |
| Diafiltration Salt Buffer (L) | - | - | - | - | - | 2048 |
| | S-424 | S-425 | S-426 | S-427 | S-428 | |
| Temperature (˚C) | 25 | 25 | 25 | 25 | 25 | |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 | |
| Protein (kg) | 0.37 | 0.34 | 0.3 | 0.05 | 0.03 | |
| Waste (L) | 344 | 8650 | 2835 | 26 | 2110 | |

| Upstream Process | Time (Days) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 125 mL Flask (Sf-9) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 250 mL Flask (BV) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 500 mL Flask (Sf-9) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 L Flask (Sf-9) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 20 L Wave (BV) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 50 L Wave (Sf-9) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| N-1 Bioreactor | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| N Bioreactor | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

*Figure 12.5.* Gantt Chart for a Single Upstream Process Run. The blue marks the Sf-9 cell seed train while the red marks the baculovirus amplification.

*Figure 12.5* shows that the upstream process is anticipated to take 24 days in total, with parallel processes of the baculovirus and Sf-9 cell scale-up occurring simultaneously over days 9 through 19. Scale-up procedure from one stage to another typically takes 3 or 5 days.

| Downstream Process | Time (Hours) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hour | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
| Depth Filtration | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HT Chromatography | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Viral Inactivation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| IEX Chromatography | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Viral Filtration | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Diafiltration | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hour | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 |
| Depth Filtration | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HT Chromatography | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Viral Inactivation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| IEX Chromatography | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Viral Filtration | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Diafiltration | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

*Figure 12.6.* Gantt Chart for a Single Downstream Process Run.

In contrast, the downstream process is significantly shorter than the upstream process. *Figure 12.6* shows that the downstream process is expected to take about three days to complete (66 hours). The majority of this time, hours 3 to 45, is taken up by the his-tag chromatography process, which requires 5 runs to complete the process. The cleaning time added between each cycle, as well as the low flow rates required to operate the column effectively, combine to make the his-tag chromatography process much longer than the other downstream units. Most of the other downstream units are about 4 hours long, with some that are long as 7 hours.

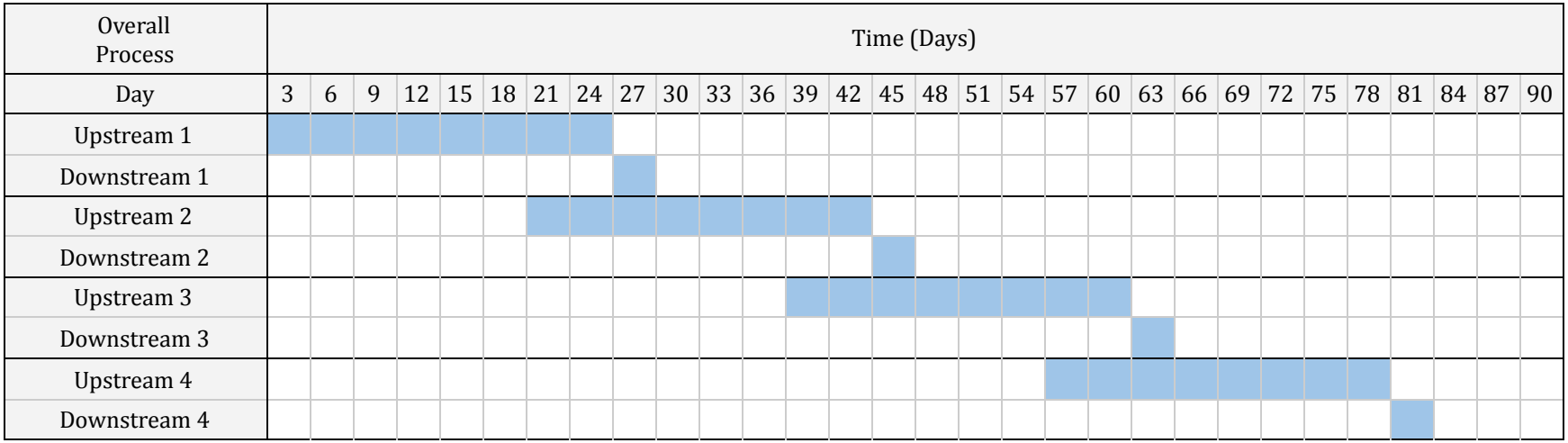


*Figure 12.7*. Gantt chart for multiple upstream and downstream process runs.

Overall, the entire upstream and downstream process takes about 27 days to complete. While we propose completing this cycle once for our project, in the event that repeated cycles are desired or necessary, we suggest that 18 days in between cycles should be sufficient time to clean equipment and account for manufacturing problems in the prior process. As shown in *Figure 12.7*, we anticipate that at least 4 production cycles can be run to completion in under 3 months (81 days), if multiple campaigns are desired.

# 13. Process Description

## 13.1. Upstream Process

### *13.1.1 Sf-9 Cell Seed Train*

*Cell Bank to Flasks*

At the start of the seed train, four frozen aliquots all with the same specified viable cell density are taken out of a cell bank. These frozen aliquots are each incubated at 27.5˚C and inoculated into the four 125 mL flasks with working volumes of 50 mL. After 5.2 days of growth in the 125 mL flasks, the contents are then each inoculated into the 500 mL flasks. The cells are grown in each flask at 27.5˚C on a shaker table.

*Flasks to WAVE Bioreactor*

After 3 days of growth in the 500 mL flasks at 27.5˚C, cell content in one of the 500 mL flasks is sent to the baculovirus preparation line for virus amplification. Cell content in the other three flasks are sent to the 5 L flasks for another 3 days of growth, after which the contents are each inoculated into the three 200 L WAVE bioreactors with working volumes of 50 L. The rocking motion of WAVE bioreactors allows efficient mixing, carbon dioxide stripping, and oxygen transfer to the cells for optimal growth.

*WAVE Bioreactor to Perfusion Bioreactor*

After 3 days of growth in the 200 L WAVE bioreactors at 27.5°C, cell content in one of the WAVE bioreactors is sent to the baculovirus preparation line for further virus amplification, and cell content with the healthiest growth in a different WAVE bioreactor is inoculated to the 500 L perfusion bioreactor. The 500 L perfusion bioreactor is different from regular batch

bioreactors in that the perfusion system allows for much higher viable cell density than normal by constantly feeding fresh medium while removing used medium.

*Perfusion Bioreactor to Production Bioreactor*

After 5 days of growth in the 500 L perfusion bioreactor at 27.5˚C, the content is finally transferred to the 2000 L batch bioreactor at 26.0˚C and infected with baculovirus at a MOI value of 0.01 for protein production. Once the content of the perfusion bioreactor is inoculated into the 2000 L bioreactor, the viable cell density is already at its maximum. Thus, cells are in the stationary phase, spending energy only for protein production and not for additional growth. After 5 days of production in the 2000 L bioreactor, the content is transferred to the downstream process.

### *13.1.2 Baculovirus Amplification*

*Cell Bank to Flasks*

After 3 days of Sf-9 cell growth in the 500 mL shake flasks at 27.5˚C, 180 mL of cell culture is sent to both 250 mL shake flasks for infection with baculovirus. The cell cultures in each shake flask will be infected with 20 mL of purchased baculovirus at an MOI value of 0.01. Baculovirus will be incubated in the cell culture for 6 days at 27.5˚C on a shaker table.

*Flasks to WAVE Bioreactor*

After 6 days of growth in the 250 mL shake flasks at 27.5˚C, lysed cells and virus will be transferred to the 20 L WAVE bioreactors and amplified there starting with an MOI of 0.01. Then, 18 L of grown cell culture will be taken from a 50 L WAVE bioreactor as described above

and will be provided for viral infection. Baculovirus will be incubated by infecting the cell culture and amplified for 5 days.

*WAVE Bioreactor to Production Bioreactor*

After 5 days of growth in the 20 L WAVE bioreactor at 27.5˚C, the incubated baculovirus will be pumped into the 2000 L production bioreactor where it will infect the cells and produce proteins. Protein will be produced in the earlier baculovirus scale-up phases as well but in marginal quantities. Once again, the MOI will be 0.01 and the incubation period will be 5 days before transfer to the downstream process.

## 13.2 Downstream Process

### *13.2.1 Depth Filtration*

Depth filtration is the first step in our downstream process. The cells have already lysed on their own before entering the depth filters, so protein of interest is extracellular. Depth filtration is efficient in that it is much more easier to develop than an intricate tangential-flow filtration system. Also, single-use disposable depth filters are economical, since the process is not run for multiple times. Fouling is not an issue because our cell density is low at 8 million cells / mL. By using a series of filters with large pore size followed by filters with small pore size, depth filtration is able to filter out large cell debris, bacteria, and some viruses. The percent recovery of depth filtration step is approximately 90%. The supernatant is sent to the his-tag chromatography for further filtration.

### *13.2.2 His-tag Chromatography*

After the supernatant leaves the depth filter, it will flow through the His-tag chromatography column. The chromatography column works by binding specifically to the genetically engineered his-residues added to the protein, allowing everything else to flow through. This step will be the most capital and time intensive step of the downstream process. The packed column will be equilibrated before material flows through. In the affinity stage, there will be five runs total, so only one fifth of the total amount of supernatant will flow through during each run. Once the supernatant flows through, the column will be washed and then eluted. The eluate will be sent to the viral inactivation phase for the next process. The flow rate will be 189 L/hr for the loading, washing, and elution stages, and 377 L/hr for the cleaning and regeneration stages. Dr. Stjin Koshari advised using a slower flow rate for the loading, washing, and elution stages to avoid protein loss. The process will be repeated 4 times. We estimate total run time will be about 2 days and each run will take about 9 hours including washing. Yield is anticipated to be about 90% with purity of around 95%.

### *13.2.3 Viral Inactivation*

Viral inactivation is necessary in order to denature the virus while keeping the protein intact. This is commonly done using low pH, specifically a pH of 3.5. The Cadence Virus Inactivation System by Pall was highly considered, as it is a fully automated system that lowers the pH using a continual elution stream and then brings the pH back up to around 8. However, Dr. Koshari recommended doing this manually, as the cost for the system is not worth it. Thus, we will be inactivating our virus in the ThermoFisher HyPerforma Single Use 2000 L Mixer. The mixer will also have a jacket in order to monitor and maintain an appropriate temperature. In

order to bring the pH down to 3.5, about 2.5% of the original volume of 1M Acetic acid should be added. In order to bring the pH back up, 5.2% of the original volume of 1M Tris base should be added [80]. Directly following this step, sterile membrane filtration will ensue and result in a 98% yield.

### *13.2.4 Ion Exchange Chromatography*

Once material is finished with the viral inactivation phase, it will flow through the ion exchange chromatography column. The chromatography column will be pre-packed and equilibrated before this run. Material will flow through at 289 L/hr and a pH above 7.24 to ensure the protein, in its anionic form binds to the anion-exchange resins. Once the protein extract has been loaded in the column, the column pH will be steadily decreased to the protein's isoelectric point at 6.24 using a pH gradient elution buffer to release the protein. The eluate will be sent on to the viral filtration stage and the column will be cleaned during that stage.We estimate that total process time without cleaning (as that can be done in parallel) is 7 hours with expected yield of 90% and purity of 85%.

### *13.2.5 Viral Filtration*

Viral filtration step is required to make sure that viral clearance of the final product is greater than log reduction value (LRV) of 6. Filters with very precise pores are used, and a pre-filter is used beforehand to prevent fouling and clogging in the actual filter. Depth filters are preferred over sterile membrane filters, since depth filters can contain more particulates, clog less, and have charge characteristics that can bind additional DNA and host cell proteins. The

percent recovery of viral filtration step is approximately 98%. The protein solution is sent to the diafiltration step for final purification.

### *13.2.6. Diafiltration*

Any small particles that are not filtered out in the viral filtration enter as feed in the diafiltration process. Membrane cassettes will be used to retain and recirculate the Spike protein while filtering out any smaller solutes, such as any small salts and sugars. A tangential flow filtration configuration will be used to minimize clogging of the membranes. A diafiltration buffer will be added to the feed tank at the same rate as the permeate leaves to maintain a constant volume in the feed tank. This buffer exchange will ensure the stability of the Spike protein for shipping. The final buffer displacement recovers 99.3% of the protein [81] and has a 99.3% reduction in salt, assuming no protein goes through the membrane and that no salts are rejected. We do not need to concentrate our Spike protein because our final protein concentration of 6.5 g/L is considered low for industry. Our final drug product will contain Spike protein in an appropriate diafiltration buffer, which will be packaged and shipped in bulk for formulation elsewhere.

# 14. Energy Balance and Utility Requirements

## 14.1 Upstream Energy Balance

The number of cells in each cell culture vessel was calculated by multiplying the working volume with the peak viable cell density. Then, the heat generated by cells was calculated by multiplying the number of cells with peak specific enthalpy of reaction. Suggested by our project author, the peak specific enthalpy of reaction for Sf-9 cells was assumed to be 40 pW/cell. *Table 14.1* shows the energy requirements for the upstream process.

*Table 14.1.* Upstream Energy Balance

| Equipment | Heat Generated by Cells (W) | Impeller Power (W) | Heat Duty of Associated Cooling Jacket (W) |
|---|---|---|---|
| 125 mL Erlenmeyer Shake Flask | 0.01 | - | - |
| 250 mL Erlenmeyer Shake Flask | 0.04 | - | - |
| 500 mL Erlenmeyer Shake Flask | 0.11 | - | - |
| 5 L Erlenmeyer Shake Flask | 1.16 | - | - |
| WAVE Bioreactor System 20/50EH | 4.98 | - | - |
| WAVE Bioreactor System 200EH | 12.44 | - | - |
| 500 L Perfusion Bioreactor | 652 | 10 | 662 |
| 2000 L Production Bioreactor | 652 | 40 | 692 |

## 14.2 Process Energy Usage

Since the cell growth and metabolism are low due to slow oxygen uptake of Sf-9 cells, low gas flow rates are sufficient, which results in very small power requirement and total work per run for the air compressors in general. Power requirement and total work per run is generally very small for peristaltic pumps as well, since high liquid flow rates are not used in pharmaceutical processes and transport. *Table 14.2* shows the energy requirements for each process.

*Table 14.2.* Process Energy Usage

| Equipment | Power Requirement | Operation Time | Work per Run |
|---|---|---|---|
| Upstream | | | |
| WAVE™ Bioreactor System 20/50EH | 0.69 kW | 5 days | 82.8 kWh |
| WAVE™ Bioreactor System 200EH | 3.6 kW | 3 days | 259.2 kWh |
| 500 L Perfusion Bioreactor | 2.4 kW | 5 days | 288 kWh |
| 2000 L Production Bioreactor | 2.4 kW | 5 days | 288 kWh |
| Downstream | | | |
| 2000 L Single-Use Mixer | 2.5 kW | 4 hrs | 10 kWh |
| Diafiltration Assembly | 4.4 kW | 7 hrs | 30.8 kWh |
| Transport | | | |
| Air Compressor (for WAVE Brx 20/50EH) | 0.12 W | 5 days | 14.94 Wh |
| Air Compressor (for WAVE Brx 200EH) | 1.84 W | 3 days | 132.62 Wh |
| Air Compressor (for 500 L Perfusion Brx) | 35.64 W | 5 days | 4277.33 Wh |
| Air Compressor (for 2000 L Production Brx) | 224.46 W | 5 days | 26934.68 Wh |
| Upstream Peristaltic Pump (BV WAVE to N Brx) | 0.50 W | 15 mins | 0.12 Wh |
| Upstream Peristaltic Pump (Sf-9 WAVE to N-1 Brx) | 0.42 W | 30 mins | 0.21 Wh |
| Upstream Peristaltic Pump (N-1 Brx to N Brx) | 6.26 W | 30 mins | 3.13 Wh |
| Downstream Peristaltic Pump (for Depth Filtration) | 15.66 W | 2 hrs | 31.32 Wh |
| Downstream Peristaltic Pump (for His-tag Column) | 0.97 W | 2 days | 46.53 Wh |
| Downstream Peristaltic Pump (for IEX Column) | 0.97 W | 7 hrs | 6.79 Wh |
| Downstream Peristaltic Pump (for Viral Filtration) | 0.54 W | 3 hrs | 1.61 Wh |
| Downstream Peristaltic Pump (for Diafiltration in) | 2.24 W | 30 mins | 1.12 Wh |
| Downstream Peristaltic Pump (for Diafiltration out) | 2.76 W | 30 mins | 1.38 Wh |
| Storage | | | |
| 200 L Media Vessel | 1.5 kW | 25 days | 900 kWh |
| 2000 L Media Vessel | 2.5 kW | 25 days | 1500 kWh |
| 3000 L Media Vessel | 2.5 kW | 25 days | 1500 kWh |
| 100 L Glucose Vessel | 0.75 kW | 10 days | 180 kWh |
| 500 L Glutamine Vessel | 1.5 kW | 10 days | 360 kWh |

## 14.3 Electricity Use

Electricity use was calculated not based on the power requirement, but based on the maximum power as a conservative method to estimate the upper limit of total electricity. *Table 14.3* shows the electricity requirements for each process.

*Table 14.3.* Electricity Use

| Equipment | Maximum Power (kW) | Operation Time | Electricity per Run (kWh) |
|---|---|---|---|
| Upstream | | | |
| WAVE™ Bioreactor System 20/50EH | 0.69 | 5 days | 82.8 |
| WAVE™ Bioreactor System 200EH | 3.6 | 3 days | 259.2 |
| 500 L Perfusion Bioreactor | 2.4 | 5 days | 288.0 |
| 2000 L Production Bioreactor | 2.4 | 5 days | 288.0 |
| Downstream | | | |
| 2000 L Single-Use Mixer | 2.5 | 4 hrs | 10.0 |
| Diafiltration Assembly | 4.4 | 7 hrs | 30.8 |
| Transport | | | |
| Air Compressor (for WAVE Brx 20/50EH) | 0.516 | 5 days | 61.9 |
| Air Compressor (for WAVE Brx 200EH) | 0.516 | 3 days | 37.2 |
| Air Compressor (for 500 L Perfusion Brx) | 0.516 | 5 days | 61.9 |
| Air Compressor (for 2000 L Production Brx) | 1.128 | 5 days | 135.4 |
| Upstream Peristaltic Pump (BV WAVE to N Brx) | 0.656 | 15 mins | 0.2 |
| Upstream Peristaltic Pump (Sf-9 WAVE to N-1 Brx) | 0.656 | 30 mins | 0.3 |
| Upstream Peristaltic Pump (N-1 Brx to N Brx) | 0.656 | 30 mins | 0.3 |
| Downstream Peristaltic Pump (for Depth Filtration) | 0.656 | 2 hrs | 1.3 |
| Downstream Peristaltic Pump (for His-tag Column) | 0.656 | 2 days | 31.5 |
| Downstream Peristaltic Pump (for IEX Column) | 0.656 | 7 hrs | 4.6 |
| Downstream Peristaltic Pump (for Viral Filtration) | 0.656 | 3 hrs | 2.0 |
| Downstream Peristaltic Pump (for Diafiltration in) | 0.656 | 30 mins | 0.3 |

| | | | |
|---|---|---|---|
| Downstream Peristaltic Pump (for Diafiltration out) | 0.656 | 30 mins | 0.3 |
| Storage | | | |
| 200 L Media Vessel | 1.5 | 25 days | 900.0 |
| 2000 L Media Vessel | 2.5 | 25 days | 1500.0 |
| 3000 L Media Vessel | 2.5 | 25 days | 1500.0 |
| 100 L Glucose Vessel | 0.75 | 10 days | 180.0 |
| 500 L Glutamine Vessel | 1.5 | 10 days | 360.0 |

## 14.4 Other Utilities

*Table 14.4* shows the chilled and purified water requirements by process.

*Table 14.4.* Other Utilities

| Equipment | Utility | Usage | Amount per Run |
|---|---|---|---|
| Upstream | | | |
| 500 L Perfusion Bioreactor | Chilled Water, 40˚F | Coolant | 2964.1 kg |
| 2000 L Production Bioreactor | Chilled Water, 40˚F | Coolant | 3314.0 kg |
| Downstream | | | |
| Depth Filtration | Purified Water, 25˚C | Buffer | 917 L |
| His-Tag Chromatography | Purified Water, 25˚C | Buffer | 7555 L |
| Ion-Exchange Chromatography | Purified Water, 25˚C | Buffer | 1023 L |
| Viral Filtration | Purified Water, 25˚C | Buffer | 26 L |
| Diafiltration | Purified Water, 25˚C | Buffer | 2116 L |
| Diafiltration | Chilled Water, 40˚F | Coolant | 996.0 kg |
| Storage | | | |
| 100 L Glucose Vessel | Purified Water, 25˚C | Diluent | 82.4 L |
| 100 L Glutamine Vessel | Purified Water, 25˚C | Diluent | 484.3 L |

# 15. Equipment List and Unit Descriptions

## 15.1 Feed Materials

### *15.1.1 Biological/Chemical Laboratory Facility*

In order to manually inoculate cells from one flask to another, a biohood is required to carry out sterile pipetting. A shaker table that is covered in a hood at 27.5˚C is needed for cells to be well-mixed in the flasks. Cost analysis was done not based on the individual biohood and shaker table, but based on the entire biological/chemical lab facility. Assuming roughly 5% of the total capital and operating costs, the biological/chemical lab cost is approximately $270,623.

### *15.1.2 Air Compressors*

Air compressor AC-101 is used to provide air from the surrounding environment for the three WAVE Bioreactor 200EH over 3 days. Air flow is needed to ensure sufficient oxygen content for cell growth as well as stripping $CO_2$ from the system. Air at 6 standard liters per minute (SLPM) will be compressed to 5.01 LPM and divided equally among the three bioreactors. Pressure will increase from 1.01 bar to 1.21 bar. The system will require 0.00247 hp to operate at the desired rate. The purchase cost is approximately $1212.50.

Air compressor AC-201 is used to provide air from the surrounding environment for the two WAVE Bioreactor 20/50EH over 5 days. Air flow is needed to ensure sufficient oxygen content for cell growth as well as stripping $CO_2$ from the system. Air at 0.8 SLPM will be compressed to 0.72 LPM and divided equally among the two bioreactors. Pressure will increase from 1.01 bar to 1.11 bar. The system will require 0.000167 hp to operate at the desired rate. The purchase cost is approximately $1212.50.

Air compressor AC-301 is used in this step to provide air from the surrounding environment for the 500 L Perfusion Bioreactor over 5 days. Air flow is needed to help ensure sufficient oxygen content (along with the 50% oxygen supply) for cell growth as well as stripping $CO_2$ from the system. Air at 25 SLPM will be compressed to 11.69 LPM before entering the perfusion bioreactor. Pressure will increase from 1.01 bar to 2.16 bar. The system will require 0.0478 hp to operate at the desired rate. The purchase cost is approximately $1212.50.

Air compressor AC-302 is used in this step to provide air from the surrounding environment for the 2000 L production bioreactor over 5 days. Air flow is needed to help ensure sufficient oxygen content (along with the 100% oxygen supply) for cell growth as well as stripping $CO_2$ from the system. Air at 150 SLPM will be compressed to 67.94 LPM before entering the production bioreactor. Pressure will increase from 1.01 bar to 2.23 bar. The system will require 0.301 hp to operate at the desired rate. The purchase cost is approximately $1095.92.

### *15.1.3 Sterile Filters*

A sterile filter is a crucial piece of equipment used to purify liquid media before it enters the sterile environment of a bioreactor. In order to ensure sterility, liquid filters need to have nominal pore size of less than 1 micron, since microorganisms are typically greater than 1 micron. All the sterile filters used in our process have a 0.8/0.2 μm double pore size, stepped barb attachments, no associated energy cost, and expected purchase price of $152.60 each. The filters are all made of polyethersulfone (PES). More specifically, each filter has the following properties:

Sterile filter SF-101a, SF-101b, and SF-101c are used to purify fresh liquid media for the Sf-9 seed trains (50 mL, 500 mL, 5 L, 50 L) coming from the main media tank used in the Sf-9 cell culture scale-up. The filters process 50 L of media total.

Sterile filter SF-101d is used to purify fresh liquid media for the fourth Sf-9 seed train (50 mL, 500 mL) coming from the main media tank used in the Sf-9 cell culture scale-up. This sterile filter processes 500 mL of media total.

Sterile filter SF-201 is used to purify fresh liquid media emerging from the 20/50EH WAVE bioreactor of the baculovirus scale-up process that is heading towards the production bioreactor. This sterile filter processes 20 L of media total.

Sterile filter SF-301 is used to purify fresh liquid media entering the 500 L, N-1 perfusion bioreactor. This sterile filter processes 450 L of media total. Note that no additional sterile filter is provided for the media entering the perfusion system itself as the perfusion system contains its own liquid filter.

Sterile filter SF-302 is used to purify fresh liquid media entering the 2000 L production bioreactor. This sterile filter processes 1473 L of media total.

Sterile filter SF-303 is used to purify fresh glucose entering the 500 L, N-1 perfusion bioreactor. This sterile filter processes 30.5 kg of aqueous glucose total.

Sterile filter SF-304 is used to purify fresh glutamine entering the 500 L, N-1 perfusion bioreactor. This sterile filter processes 11.3 kg of aqueous glutamine total.

Sterile filter SF-305 is used to purify fresh base media entering the 500 L, N-1 perfusion bioreactor. This sterile filter processes 1.5 L of media total.

Sterile filter SF-306 is used to purify fresh antifoam entering the 500 L, N-1 perfusion bioreactor. This sterile filter processes 8.1 L of media total.

Sterile filter SF-307 is used to purify fresh glucose entering the 2000 L production bioreactor. This sterile filter processes 21.6 kg of aqueous glucose total.

Sterile filter SF-308 is used to purify fresh glutamine entering the 2000 L production bioreactor. This sterile filter processes 5.15 kg of aqueous glutamine total.

Sterile filter SF-309 is used to purify fresh base entering the 2000 L production bioreactor. This sterile filter processes 1.1 L of base total.

Sterile filter SF-310 is used to purify fresh antifoam entering the 2000 L production bioreactor. This sterile filter processes 6 L of antifoam total.

### *15.1.4 Media Prep Vessels*

The media prep vessels are crucial features in providing storage for the media until it can be used in the shake flasks and bioreactors. While the stainless steel media and its storage vessel must be clean, they are not inherently sterile, which is why sterile filters are used for all material emerging from the storage tank. The storage vessels also have small energy requirements originating from monitors and mixers present within the system.

The 200 L media storage tank (M-101) is used to hold media for all the Sf-9 seed trains in the initial scaleup phase (50 mL, 500 mL, 5 L, and 50 L). It has an energy requirement of 2.01 hp. It will hold about 151 L of media, and 200 L is the standard production size. The purchasing cost is $4000.00.

The 3000 L storage tank (M-301) is used to hold all the media required for the 500 L, N-1 perfusion bioreactor combined with its perfusion system. It has an energy requirement of 3.4 hp. It will hold about 2650 L of media, and 3000 L is the standard production size for that quantity. The purchasing cost is about $12,421.00.

The 2000 L storage tank (M-302) is used to hold all the media required for the 2000 L production bioreactor. It has an energy requirement of 3.4 hp. It will hold about 1473 L of media, and 2000 L is the standard production size for that quantity. The purchasing cost is about $10,210.00.

### *15.1.5 Oxygen Tanks*

Oxygen transfer is a crucial determinant of cell growth in the bioreactors. As bioreactors increase in size, oxygen transfer through diffusion from the atmosphere is no longer sufficient, requiring the specific addition of concentrated oxygen into the bioreactor. Oxygen tanks provide that extra oxygen supply to the bioreactor. The oxygen tanks don't have energy requirements. Oxygen will be purchased in large quantities for the entire system at a *total* cost of $7186.60 including the tanks to hold it. Oxygen will come in both 50% and 100% concentrations to accommodate the needs of each bioreactor. No added filtration will be required for the oxygen as air filters are built into the bioreactors.

Oxygen tank (O-301) provides 82.4 kg of 50% oxygen supply to the perfusion bioreactor. The oxygen will first be compressed before entering the bioreactor.

Oxygen tank (O-302) provides 61.8 kg of 100% oxygen supply to the perfusion bioreactor. The oxygen will first be compressed before entering the bioreactor.

### *15.1.6 Glucose Vessel*

Glucose is a critical nutrient used by Sf-9 cells for cell metabolism. While in low cell concentrations and in small volumes the Sf-900 II SFM media contains sufficient nutrients for the cells to grow. At the larger scale of the bioreactors, additional nutritional elements are required to provide continued fuel for cell metabolism and production. The stainless steel glucose vessel (GC-301) used to hold the glucose will be 100 L and contain 75 kg of glucose

solubilized in purified water. The glucose vessel has a mixer and other electrical implements that require about 1 hp of electricity. The vessel has a purchase cost of $2000.00.

### *15.1.7 Glutamine Vessel*

In addition to glucose, glutamine is another crucial metabolite for cell growth and will be contained in the stainless steel 500 L vessel (GT-301) to provide glutamine to the perfusion and production bioreactor. The vessel will carry 20 kg of glutamine solubilized in purified water. This glucose vessel has a mixer and other electrical implements that require about 2 hp of electricity to operate. The vessel has a purchase cost of $3000.00.

### *15.1.8 Base Container*

Base is a necessary component used to counteract the acidity of the $CO_2$ produced by the Sf-9 cells in the perfusion and production bioreactor. At high levels of $CO_2$ production in the perfusion and production bioreactor, the pH will drop and cell growth can be stunted. Base can be added to increase the pH to optimal value. As the volume of the base required is small, no additional tank is needed beyond the 4 L container (B-301), in which the base is purchased. A total of 2.6 L will actually be used of this total amount. No energy requirements are associated with this container, and the purchase cost of this amount of base is $105.45.

### *15.1.9 Antifoam Vessel*

As cell growth, mixing, and sparging of gas occur in the bioreactors, a risk of foam forming at the top of the perfusion or production bioreactor is a distinct possibility. As the foam collapses, it can create pressure waves that are harmful to the cells in the bioreactor. In order to

prevent this, anti-foam is added and will be stored in (AF-301). The vessel itself is 20 L, which is more than sufficient to store the required 14.1 L of antifoam needed for the system. The antifoam vessel has no associated energy requirement and has a purchase cost of $269.90.

## 15.2 Upstream Processes

### *15.2.1 Erlenmeyer Shake Flasks*

Erlenmeyer shake flasks are generally made of plastic or glass material and have either baffled or plain bottom. For our project, we chose polycarbonate flasks that have been sterilized in advance by gamma radiation. Although baffled types have depressions in the wall that allow better oxygen supply, plain types were chosen for our purposes because the growth rate of Sf-9 cells is slow. The flasks all have a microporous vent lid that keeps the content sterile and permits passage of gas. Room air is sufficient for enough oxygen to flow in and carbon dioxide to flow out. All flasks are maintained at 27.5˚C on a shaker table.

The four 125 mL flasks (F-101) are used to grow Sf-9 cells from 4.05 million cells to 249 million cells over 5.2 days of batch time. For these F-101 flasks, because the working volume is only 50 mL, the shaker table is shaked slowly to prevent evaporation. The purchase cost of F-101 is $15.70 each.

The four 500 mL flasks (F-102) are used to grow Sf-9 cells from 249 million cells to 2.68 billion cells over 3 days of batch time. The working volume of the F-102 flasks is 500

mL. Out of the four F-101 flasks, one of them is sent to the baculovirus preparation line for virus amplification. The purchase cost of F-102 is $27.10 each.

The three 5 L flasks (F-103) are used to grow Sf-9 cells from 2.68 billion cells to 28.9 billion cells over 3 days of batch time. The working volume of the F-103 flasks is 5 L. The purchase cost of F-103 is $166.00 each.

The two 250 mL flasks (F-201) are used to amplify baculovirus from 20 million viruses to 2 billion viruses by infecting 2 billion cells over 6 days of batch time. The working volume of the F-201 flasks is 200 mL. The purchase cost of F-201 is $17.70 each.

### *15.2.2 WAVE Bioreactors*

For the WAVE bioreactors, AC-101, AC-201, AC-301, and AC-302 flow air through the headspace in the WAVE cellbags. Because the viable cell density is not high in the WAVE bioreactors, air flow needs to be slow, otherwise carbon dioxide will be over-stripped by the fast air flow.

The three WAVE Bioreactor 200EH (W-101) units are used to grow Sf-9 cells from 28.9 billion cells to 311 billion cells over 3 days of batch time. The working volume of W-101 bioreactors is 50 L, the rocking speed is 25 rocks/min, and the rocking angle is 8˚. The aeration rate used for the W-101 units is 2 LPM, as suggested by the user guide manual from GE Healthcare® [82]. Out of the three W-101 units, one of them is sent to the baculovirus preparation line for further virus amplification, and only one of the other two

W-101 units is sent to the 500 L perfusion bioreactor for further cell growth. The material of construction for the W-101 bioreactors is stainless steel, the maximum electrical power requirement is 0.69 kW, and the purchase cost is $16,000 each. Three 200 L cell culture bags, which cost $1288.64 each, need to be purchased for disposable single usage.

The two WAVE Bioreactor 20/50EH (W-201) units are used to amplify baculovirus from 2 billion viruses to 200 billion viruses by infecting 200 billion cells over 5 days of batch time. The working volume of the W-201 bioreactors is 20 L, the rocking speed is 20 rocks/min, and the rocking angle is 7˚. The aeration rate used for the W-201 units is 0.4 LPM, as suggested by the user guide manual from GE Healthcare® [82]. Out of the two W-201 bioreactors, only one of them is sent through SF-201 to allow baculovirus to pass but capture cell debris, and then pumped to the 2000 L production bioreactor for infection and protein expression. The material of construction for the W-201 units is stainless steel, the maximum electrical power requirement is 3.6 kW, and the purchase cost is $4,000 each. Two 50 L cell culture bags, which cost $322.16 each, need to be purchased for disposable single usage.

### *15.2.3 Perfusion Bioreactor and System*

The perfusion bioreactor (PBrx-301) with the perfusion system (PS-301) is used to grow Sf-9 cells from 311 billion cells to 16.3 trillion cells over 5 days of perfusion time. The working volume of PBrx-301 is 500 L. Liquid media, nutrients, base, and antifoam flow through SF-6/8~11 before entering PBrx-301. PS-301 has its own sterile filter for replenishing 2200 L of

fresh media into PBrx-301, allowing the maximum VCD as well as number of cells to increase 4-fold. PBrx-301 has its own built-in sterile filters for gas materials, i.e. air and oxygen.

PBrx-301 is equipped with one pitched blade impeller with three blades. The impeller is used mainly for homogenizing temperature, pH, and liquid composition. The impeller can also aid in preventing cells from settling to the bottom, but because the cells typically sink at a very slow rate, the sparger alone is usually sufficient. The agitation speed of the impeller is 101 RPM, which leads to a mixing time of 27 seconds to reach 95% homogeneity, as shown in Section 25.4 calculations.

PBrx-301 is also equipped with a dual sparger-configuration with one porous frit sparger and one drilled hole sparger. Oxygen supply at 50% oxygen is sparged through the frit sparger at 8 SLPM; air is used for carbon dioxide stripping, which is sparged through the drilled hole sparger at 25 SLPM. The air flow rate out was assumed to be the same as air flow rate in at 25 SLPM in order to find out the maximum size of vent filter needed, as well as the permit for maximum air vent.

The heat generated by Sf cells is 652 W and that by the impeller is 10 W, which means that PBrx-301's cooling jacket (CJ-301) needs to use a total of 662 W to control the temperature to 27.5˚C. PBrx-301 has its own pH probe that monitors the change in pH level due to cellular respiration, and if the pH becomes too low, 1 M sodium hydroxide is added as base to maintain the pH level at 6.3.

The material of construction for PBrx-301 is stainless steel, the maximum electrical power requirement is 2.4 kW The purchase cost is $91,473.39 for PBrx-301 and $18,560.00 for PS-301. One 500 L BioProcess Container (BPC), which costs $4,453.99, needs to be purchased for disposable single usage. The 500 L BPC has been sterilized in advance by gamma radiation.

### *15.2.4 Production Bioreactor*

The production bioreactor (Brx-301) is used to express 3.75 kg of spike protein over 5 days of batch time by infecting 16.3 trillion Sf-9 cells with 200 billion baculovirus from one of the two W-201 units. Taking into consideration the 30% yield loss in the downstream process, the amount of expressed spike protein is 50% more than what is needed for 500 million doses. The working volume of Brx-301 is 2000 L. Liquid media, nutrients, base, and antifoam flow through SF-7/12~15 before entering the Brx-301.

Brx-301 is equipped with one pitched blade impeller with three blades as well, but with a larger size. The impeller is, again, used mainly for homogenizing temperature, pH, and liquid composition. The agitation speed of the impeller is 75 RPM, which leads to a mixing time of 36 seconds to reach 95% homogeneity, as shown in Section 25.4 calculations.

Brx-301 is also equipped with a dual sparger-configuration with one porous frit sparger and one drilled hole sparger. Oxygen supply at 100% oxygen is sparged through the frit sparger at 6 SLPM; air is used for carbon dioxide stripping, which is sparged through the drilled hole sparger at 150 SLPM. The air flow rate out was assumed to be the same as air flow rate in at 150 SLPM in order to find out the maximum size of vent filter needed, as well as the permit for maximum air vent.

The heat generated by Sf cells is 652 W and that by the impeller is 40 W, which means that Brx-301's cooling jacket (CJ-302) needs to use a total of 692 W to control the temperature to 26˚C. Brx-301 also has its own pH probe that monitors the change in pH level due to cellular respiration, and again if the pH becomes too low, 1 M sodium hydroxide is added as base to maintain the pH level at 6.3.

The material of construction for Brx-301 is stainless steel, the maximum electrical power requirement is 2.4 kW, and the purchase cost is $214,065.99. One 2000 L BPC, which costs $10,233.13, needs to be purchased for disposable single usage. The 2000 L BPC has been sterilized in advance by gamma radiation.

## 15.3 Downstream Processes

### *15.3.1 Depth Filtration*

The depth filtration unit (DeF-401) filters out large cell debris, bacteria, and some viruses in feed stream S-401 that comes from Brx-301 by using a two-stage depth filter train. Traditionally, non-synthetic HC filters have been used, but these filters have leachables that impact the product quality. Thus, industries are leaning towards using synthetic SP filters, which have similar functionality but no leachables. The 26 D0SP filters and 13 X0SP filters from Millipore are used in our two-stage depth filter train. The D0SP filters have a pore size of 10 μm that filter out large sized cell debris, and the X0SP filters have a pore size of 0.1 μm that filter out bacteria and some viruses. The size of our protein of interest is around 180 to 200 kDa [1][83], which is much smaller than the pore size of X0SP filters and allows the protein to pass through. A process-scale pod (PSP) device format of two 2-rack holders, which can process up to 20 filters each, is used. One of the 2-rack holders holds 20 D0SP filters, and the other one holds 6 D0SP filters and 13 X0SP filters.

After setting up the PSP device, 459 L of buffer (purified water) is added for wetting. Then, the 2000 L harvest batch with 3.75 kg of protein enters the depth filters at a flow rate of 2002 L/hr. The titer from the harvest batch is 1.875 g of protein/L medium, which is suitable for

entry into depth filtration. Next, 75% of the buffer (344 L of buffer) is discarded, and the remaining 25% of the buffer (115 L of buffer) is obtained with the 2000 L feed by flushing the depth filters again using 459 L of buffer. Additional 251 L of volume is recovered after air blowdown, totaling a final volume of 2366 L for processing in the next downstream operation unit. The time required for depth filtration is roughly 1 hour of feed process time and 1 hour of wetting/flushing time, which adds up to 2 hours. The percent yield of depth filtration is around 90%, which means 3.38 kg of protein is recovered in exit stream S-402 for further downstream processing.

The material of construction for the PSP 2-rack holders is 316L stainless steel. D0SP filters are made of non-woven, silica filter aid / polyacrylic fiber pulp, and X0SP filters are made of silica filter aid / polyacrylic fiber pulp. The purchase cost is $52,090 for the 2-rack process pod holder, $834 for each of the D0SP filters, and $1,050 for each of the X0SP filters.

### *15.3.2 His-tag Chromatography*

Before the protein solution from DeF-401 is added to the Cytiva Chromaflow Chromatography Column (HC-401), which has a diameter of 400 mm and a height of 400 mm, the column will be packed using 33.75 L of the HisPur™ Cobalt Superflow Agarose resin. The column will then be equilibrated by using 10 column volumes, or 503 L, of an equilibration buffer flowed through at 300 cm/hr (377 L/hr), and only then 473 L of the protein extract from S-404 will flow through the column. As five runs will be conducted, only one fifth of the loading material i.e. will need to flow through the column per run, hence 473 L. The protein extract will be run through at a rate of 150 cm/hr or 188.5 L/hr when sizing appropriately for the column. Once all the material has run through, 10 column volumes, 503 L, of the washing buffer will be

applied to the column at 188.5 L/hr. 10 column volumes of the elution buffer will next be applied to the column at 188.5 L/hr and the eluate will be transferred to the 2000 L single-use mixer (VI-401) in viral inactivation stage. The column will then be washed with 10 column volumes of a washing buffer, followed by 10 column volumes of a regeneration buffer. Both of these cleaning buffers can be run faster at 377 L/hr, as there is no concern for protein loss.

The process will repeat from the equilibration buffer phase onwards until all protein extract has been processed. Total run time will be approximately 2 days and each run will take about 9 hours including washing. Yield is anticipated to be about 90% with purity of around 95% [84], which means that about 3.04 kg of protein exits the stream S-405. The purchase cost of his-tag chromatography column is approximately $76,000.

### *15.3.3 Viral Inactivation and Filtration*

The viral inactivation step will denature the virus particles remaining in stream [S-405]. The ThermoFisher HyPerforma Single Use 2000 L Jacketed Mixer (VI-401) will be used. The tank is 135 cm in diameter and 185 cm tall. The feed volume will be 1257 L. First, 31.4 L of 1 M acetic acid will be added to VI-401 in stream S-420 to bring the pH down to 3.5. The average acid-addition flow rate will be 30 L/hr, with a faster slow rate at the start and slower flow rate at the end. This will take approximately 1 hour. Then, a holding time of 1 hour will ensue. Next, 65.3 L of 1 M tris base will be added through stream S-420 at an average flow rate of 60 L/hr, done so in the same fashion as the acid-addition with a fast start and slow finish. The base can be added at double the flow rate as the acid because it is not as strong as the acid, according to Dr. Koshari. The base-addition will therefore take about 1 hour. The purchase cost for VI-401 is estimated to be about $160,550. The electrical requirement for the single-use mixer is 2.5 kWh.

The next step will be a sterile membrane filtration. We will use the Millipore Express SHF Hydrophilic Filter with Opticap XL 10 Capsule (MF-401), which has a surface area of 0.54 $m^2$ and a flux of 20 ml/min-$cm^2$. This correlates to 200L/min-$m^2$, which in turn yields a flow rate of 108 L/min. This is quite high, as we believe that the data available on small scale flux would not directly correlate. Therefore, we assume the flow rate to be about 30L/hr and will take at most 1 hour to complete. The yield is 98% with 2.98 kg of protein leaving stream S-406. The cost for the sterile membrane filter is $970. The total time for viral inactivation and sterile membrane filtration will be 4 hours.

### *15.3.4 Ion Exchange Chromatography*

The Millipore QuickScale Biochromatography Column (IEX-401) has a diameter of 350 mm and a height of 550 mm. It will be packed with 21.3 L of resin and equilibrated with 5 column volumes of equilibration buffer at 289 L/hr. Then, material in S-408 will be run through IEX-401 all at once at 300 cm/hr or 289 L/hr. Once the protein extract has run through the column, the elution buffer will be applied to change the pH in the column, and gradually release the protein. The 370 L eluate will be collected and sent on to VF-401. The column will then be washed and regenerated for another cycle.

Total process time without cleaning (which could be done in parallel) is anticipated to be 7 hours with expected yield of 90% [85] and purity of 85% [86], which means that about 2.68 kg of protein exit stream S-409. The purchase cost of IEX-401 is approximately $43,320.

### *15.3.5 Viral Filtration*

The viral filtration unit (VF-401) removes viruses in feed stream S-411 coming from IEX-401, and the Viresolve® adsorptive depth prefilter is used with Magnus 2.1 filter for the VF-401. These filters together have been shown to provide log reduction value greater than 4 for 28 nm sized bacteriophage challenged at $10^7$ pfu/cm$^2$ [87]. The nucleocapsids of baculovirus in our solution average 30 to 70 nm in diameter and 200 to 400 nm in length [88], which suggests that final log reduction value of 6 for viral clearance is very likely to be achieved, since some viruses have already been filtered out in the depth filtration and chromatography steps.

After setting up the prefilter and Magnus 2.1 device, 26 L of purified water is added for wetting. Then, the 370 L with 2.68 kg of protein from the ion exchange chromatography column enters the viral filters at a flow rate of 155.3 L/hr. The titer load entering the viral filtration step is 7.23 g of protein/L medium, which corresponds to a mass throughput of 8.4 kg/m$^2$ as shown in the Viresolve® Pro Solution Performance Guide [89]. Then, 26 L of 50 mM acetate buffer is added last for flushing, totaling a final volume of 396 L for processing in the next downstream operation unit. The time required for viral filtration is around 3 hours with 98% yield, which means 2.63 kg of protein is leaving stream S-412 for further downstream processing.

The adsorptive depth prefilter is composed of diatomaceous earth (DE), cellulose fibers, and a binder containing cationic imine groups. Materials of construction for the Magnus 2.1 filter are polyethersulfone (PES) for the membrane, polyvinylidene fluoride (PVDF) for the housing, and silicone for the gaskets. The purchase cost is $10,270 for the Magnus 2.1 filter, and $1,340 for the adsorptive depth prefilter.

### *15.3.6 Diafiltration*

The diafiltration unit (DiF-401) retains the Spike protein and filters out any small particles from the feed stream S-414 pumped from the viral filtration outlet stream S-413. Since the Spike protein is 180 kDa in size [1][83], a nominal molecular weight limit of 30 kDa was chosen for the membrane cassettes using Millipore supplier guidelines to retain the protein and filter out any smaller solutes. The Millipore® Ultracel® membrane made of regenerated cellulose was chosen due to its extremely low protein binding, since our protein product is highly valuable. This membrane line provides the highest product recovery from Millipore® and is ideal for solutions with protein concentrations below 20 g/L. Three 1.14$m^2$ Millipore® Pellicon® cassette filters are stacked and run in parallel for a total membrane area of 3.42$m^2$, accounting for a 20% safety factor for the membrane area [90].

The 400 L feed stream S-414 contains 6.6 g Spike protein / L buffer. The process will be run twice, each at 200 L of feed and 5 diavolumes to remove 99.3% of smaller solutes [90][91]. This means 1000 L of diafiltration buffer will be used each run, totaling to 2000 L of buffer required for two runs entering in S-423 and exiting in S-428. The diafiltration buffer will consist of 150mM NaCl and 2.5mM $NaPO_4$ in WFI (water for injection), at a pH between 6.8 and 7.2, based on a diafiltration buffer used in the development of a SARS recombinant spike protein vaccine [92]. Each run takes 3.5 hours at a feed flow of 4 L/min and a permeate flow of 4.7 L/min, totaling to 7 hours of combined processing time. The final buffer displacement will recover 99.3% of the protein [81] using 3 L of buffer for each run, totaling to a 6 L dilution using additional buffer. The final product contains 6.5 g Spike protein / L buffer, which totals to a final mass of 2.6 kg Spike protein. This is more than the 2.5 kg of protein required to produce 500 million doses, which leaves room for unforeseen losses in between processes.

Based on energy balance calculations, the heat generated by the mixing and pumping of liquid will lead to an 8°C increase in temperature, which will be brought back down to the operating temperature of 25°C using a heating jacket that comes with the system. The system requires 4.4 kW of electrical utility to run and 3.4 kW to cool. After consultation with Dr. Koshari, the diafiltration system was estimated to be between $200,000 and $400,000 as a single-use operation based on the $1.2 million cost of a stainless steel version. To be conservative, this system was estimated to cost about $300,000.

## 15.4 Storage and Transfer

### *15.4.1 Peristaltic Pumps*

Pumps are used to transport liquid between vessels. Although centrifugal pumps can handle very high flow rate and high discharge pressure, peristaltic pumps are favored over centrifugal pumps in that peristaltic pumps preserve sterility, since fluid never touches the equipment itself and only flows through peristaltic tubes. These peristaltic pumps are positive displacement pumps that are capable of emptying all liquid with air, which means that there is no dead volume waste. They are also specifically designed to be compatible with the B/T 87 and B/T 91 series of tubing.

Pump (PP-301) is used to transport the feed stream (S-105) from the WAVE bioreactor 200EH (W-101a) to the perfusion bioreactor (PBrx-301). PP-301 transports 50 L at 1.67 LPM while increasing the pressure from 1.21 bar to 1.36 bar. It requires 0.00056 hp to operate and has a purchasing cost of $7390.00.

Pump (PP-302) is used to transport the feed stream (S-302) from the 500 L N-1 perfusion bioreactor (PBrx-301) to the 2000 L production bioreactor (Brx-301). PP-302 transports 500 L at 16.67 LPM while increasing the pressure from 1.04 bar to 1.54 bar. It requires 0.0084 hp to operate and has a purchasing cost of $7390.00.

Pump (PP-303) is used to transport the feed stream (S-203) from the WAVE bioreactor 20/50EH (W-201a) to the 2000 L production bioreactor (Brx-301). This pump transports 20 L at 1.33 LPM while increasing the pressure from 1.11 bar to 1.54 bar. The pump requires 0.00067 hp to operate and has a purchasing cost of $7390.00.

Pump (PP-401) is used to transport the protein extract (S-304) from the 2000 L production bioreactor (Brx-301) through the depth filtration (DeF-401) to the 3000 L storage tank (ST-401). PP-401 transports 2000 L at 33.37 LPM while increasing the pressure from 1.04 bar to 3.14 bar. It requires 0.021 hp to operate and has a purchasing cost of $7390.00.

Pump (PP-402) is used to transport the protein extract (S-403) from the 3000 L storage tank (ST-401) through the his-tag chromatography column (HC-401) to the 2000 L single use mixer (VI-401) at the viral inactivation tank. PP-402 transports 2366 L at 3.13 LPM while increasing the pressure from 1.04 bar to 2.03 bar. It requires 0.0013 hp to operate and has a purchasing cost of $7390.00.

Pump (PP-403) is used to transport the protein extract (S-406) from the 2000 L single use mixer (VI-401) at the viral inactivation tank through the ion-exchange chromatography column (IEX-401) to the 500 L storage tank (ST-402). PP-403 transports 1353 L at 4.82 LPM while increasing the pressure from 1.04 bar to 3.44 bar. It requires 0.0013 hp to operate and has a purchasing cost of $7390.00.

Pump (PP-404) is used to transport the protein extract (S-410) from the first 500 L storage tank (ST-402) through the viral filtration unit (VF-401) to the second 500 L storage tank (ST-403). PP-404 transports 370.4 L at 2.59 LPM while increasing the pressure from 1.04 bar to 3.14 bar. It requires 0.00072 hp to operate and has a purchasing cost of $7390.00.

Pump (PP-405) is used to transport the protein extract (S-413) from the second 500 L storage tank (ST-403) to the diafiltration unit (DiF-401). PP-405 transports 396 L at 13.33 LPM while increasing the pressure from 1.04 bar to 1.28 bar. It requires 0.0030 hp to operate and has a purchasing cost of $7390.00.

Pump (PP-406) is used to transport the protein extract (S-415) from the diafiltration unit (DiF-401) to the third 500 L storage tank (ST-404). PP-406 transports 402 L at 13.33 LPM while increasing the pressure from 1.01 bar to 1.09 bar. It requires 0.0037 hp to operate and has a purchasing cost of $7390.00.

### *15.4.2 Peristaltic Tubes*

The peristaltic tubes used in the process are a crucial part of ensuring product sterility during transfer between production phases. The peristaltic tubing used is made of platinum-cured silicone to ensure compatibility with pharmaceutical grade products as well as a sterile environment. The tubing is also sufficiently flexible to withstand the compressions occurring during the pumping. Straight hose barb unions will be used to connect the tubing to ensure sterile connections. Each unit of tubing is 10 ft long. However, in order to ensure sufficient spacing between pieces of equipment, 20 ft (i.e. 2 pieces of tubing) will be used between equipment items. The tubing itself has no associated energy costs although energy will be required to pump material through. The tubing itself is designed to be smooth to minimize flow obstruction. Due to differences in the required flow rate throughout the process, two tubing sizes will be used: the B/T 87 and the B/T 91.

The B/T 87 has an internal diameter of ½ inch and is used for smaller flow rates as it has a flow rate capacity of up to 18.9 LPM. It can withstand up to 2.4 bar of pressure. It is used for all tubing requirements except for the tubing between the production phase bioreactor and the first storage tank. The tubing has a purchase cost of $484.00/10 ft.

The B/T 91 has an internal diameter of ¾ inch and is used for larger flow rates as it has a flow rate capacity of up to 42 LPM. It can withstand up to 2.06 bar of pressure. It is used for the tubing between the production phase bioreactor and the first storage tank. $691.00/10 ft.

### *15.4.3 Storage Vessels*

Storage vessels are large stainless steel containers that are meant to hold material until it can be transferred to another stage of the process. These containers are built to be sturdy and stable in the presence of biological matter.

The material coming out of the depth filtration step (S-402) requires a large storage vessel that can hold all protein extract mixed with the depth filtration buffer. Storage Tank 1 (ST-401) is 3000 L to hold all 2400 L of solution and to be of a standard production size. ST-401 is made out of stainless steel for its durability. It will not have any associated energy requirements and has a purchase price of $12,400.

The 370 L of protein-containing elution buffer flowing from the ion-exchange chromatography step (S-409) will be sent to Storage Tank 2 (ST-402) for storage before continuing through to the viral filtration stage (VF-401) at a different flow rate. ST-402 is 500 L to ensure the vessel is of standard production size. It will not have any associated energy requirements and has a purchase price of $1,500.

The 395 L of protein extract flowing from the viral filtration system (S-412) will flow into Storage Tank 3 (ST-403) before processing in the diafiltration stage (DiF-401). ST-403 is 500 L to ensure the vessel is of standard production size. It will not have any associated energy requirements and has a purchase price of $1,500.

The 402 L of protein extract flowing from the diafiltration system (S-415) will flow into Storage Tank 4 (ST-404) until formulation can be carried out. ST-404 is 500 L to ensure the vessel is of standard production size. It will not have any associated energy requirements and has a purchase price of $1,500.

# 16. Specification Sheets

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Corning® 125 mL Erlenmeyer Shake Flasks (with Vent Cap) | | | |
| **Equipment** | ID | F-101a/b/c/d | |
| | Quantity | 4 | |
| | Function | Grow cells to VCD of 4.98 million cells / mL | |
| | Operation | Batch, 5.2 days of growth | |
| **Materials** | | In | Out |
| | Feed (with Sf-9 Cells) | 1 mL | 50 mL |
| | Media (Sf-900™ II SFM) | 49 mL | - |
| **Design Data** | Flask Material | Polycarbonate | |
| | Cap Material | Polypropylene | |
| | Bottom Style | Plain | |
| | Cap Style | Vent | |
| | Sterile | Yes | |
| | Feature | Disposable | |
| | Temperature | 27.5 ± 0.5 ˚C | |
| | pH | 6.3 ± 0.1 | |
| | Pressure | 1.01 bar | |
| | Height | 4.54 in (with cap)<br>4.45 in (w/o cap) | |
| | Base Diameter | 2.58 in | |
| | Working Volume | 50 mL | |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost is $15.70 each; flask is sterilized by gamma radiation and certified non-pyrogenic. | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Corning® 250 mL Erlenmeyer Shake Flasks (with Vent Cap) | | | |
| **Equipment** | ID | F-201a/b | |
| | Quantity | 2 | |
| | Function | Grow 2 million baculoviruses to 100-fold | |
| | Operation | Batch, 6 days of growth | |
| **Materials** | | In | Out |
| | Feed (with Baculovirus) | 20 mL | 200 mL |
| | Media with Sf-9 Cells | 180 mL | - |
| **Design Data** | Flask Material | Polycarbonate | |
| | Cap Material | Polypropylene | |
| | Bottom Style | Plain | |
| | Cap Style | Vent | |
| | Sterile | Yes | |
| | Feature | Disposable | |
| | Temperature | 27.5 ± 0.5 ˚C | |
| | pH | 6.3 ± 0.1 | |
| | Pressure | 1.01 bar | |
| | Height | 5.50 in (with cap)<br>5.42 in (w/o cap) | |
| | Base Diameter | 3.28 in | |
| | Working Volume | 200 mL | |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost is $17.70 each; flask is sterilized by gamma radiation and certified non-pyrogenic. | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Corning® 500 mL Erlenmeyer Shake Flasks (with Vent Cap) | | | |
| **Equipment** | ID | F-102a/b/c/d | |
| | Quantity | 4 | |
| | Function | Grow cells to VCD of 5.36 million cells / mL | |
| | Operation | Batch, 3 days of growth | |
| **Materials** | | In | Out |
| | Feed (with Sf-9 Cells) | 50 mL | 500 mL |
| | Media (Sf-900™ II SFM) | 450 mL | - |
| **Design Data** | Flask Material | Polycarbonate | |
| | Cap Material | Polypropylene | |
| | Bottom Style | Plain | |
| | Cap Style | Vent | |
| | Sterile | Yes | |
| | Feature | Disposable | |
| | Temperature | 27.5 ± 0.5 ˚C | |
| | pH | 6.3 ± 0.1 | |
| | Pressure | 1.01 bar | |
| | Height | 7.05 in (with cap)<br>6.96 in (w/o cap) | |
| | Base Diameter | 3.99 in | |
| | Working Volume | 500 mL | |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost is $27.10 each; flask is sterilized by gamma radiation and certified non-pyrogenic. | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Corning® 5 L Erlenmeyer Shake Flasks (with Vent Cap) | | | |
| **Equipment** | ID | F-103a/b/c | |
| | Quantity | 3 | |
| | Function | Grow cells to VCD of 5.78 million cells / mL | |
| | Operation | Batch, 3 days of growth | |
| **Materials** | | In | Out |
| | Feed (with Sf-9 Cells) | 0.5 L | 5 L |
| | Media (Sf-900™ II SFM) | 4.5 L | - |
| **Design Data** | Flask Material | Polycarbonate | |
| | Cap Material | Polypropylene | |
| | Bottom Style | Plain | |
| | Cap Style | Vent | |
| | Sterile | Yes | |
| | Feature | Disposable | |
| | Temperature | 27.5 ± 0.5 ˚C | |
| | pH | 6.3 ± 0.1 | |
| | Pressure | 1.01 bar | |
| | Height | 11.33 in (with cap)<br>11.24 in (w/o cap) | |
| | Base Diameter | 9.05 in | |
| | Working Volume | 5 L | |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost is $166.00 each; flask is sterilized by gamma radiation and certified non-pyrogenic. | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Cytiva® WAVE™ Bioreactor System 20/50EH | | | |
| **Equipment** | ID | W-201a/b | |
| | Quantity | 2 | |
| | Function | Grow 2 billion baculoviruses to 100-fold | |
| | Operation | Batch, 5 days of growth | |
| **Materials** | | In | Out |
| | Feed (with Baculovirus) | 0.2 L | 20 L |
| | Media with Sf-9 Cells | 19.8 L | - |
| | Air | 2.2 kg (0.4 LPM) | 2.2 kg (0.4 LPM) |
| **Design Data** | Material | Stainless Steel | |
| | Temperature | 27.5 ± 0.5 ˚C | |
| | pH | 6.3 ± 0.1 | |
| | Pressure | 1.11 bar | |
| | Length | 0.775 m | |
| | Width | 0.700 m | |
| | Height | 0.254 m | |
| | Weight | 15.5 kg | |
| | Working Volume | 20 L | |
| | Rocking Speed | 25 rocks/min | |
| | Rocking Angle | 8 degrees | |
| **Utilities** | Voltage | 110/230 VAC | |
| | Frequency | 50/60 Hz | |
| | Current | 6/3 A | |
| **Comments**: Purchase cost is $4000.00 each | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Cytiva® WAVE™ Bioreactor System 200EH | | | |
| **Equipment** | ID | W-101a/b/c | |
| | Quantity | 3 | |
| | Function | Grow cells to VCD of 6.22 million cells / mL | |
| | Operation | Batch, 3 days of growth | |
| **Materials** | | In | Out |
| | Feed (with Sf-9 Cells) | 5 L | 50 L |
| | Media (Sf-900™ II SFM) | 45 L | - |
| | Air | 11.0 kg (2 LPM) | 11.0 kg (2 LPM) |
| **Design Data** | Material | Stainless Steel | |
| | Temperature | 27.5 ± 0.5 ˚C | |
| | pH | 6.3 ± 0.1 | |
| | Pressure | 1.21 bar | |
| | Length | 1.852 m | |
| | Width | 1.096 m | |
| | Height | 1.120 m | |
| | Weight | 350 kg | |
| | Working Volume | 50 L | |
| | Rocking Speed | 20 rocks/min | |
| | Rocking Angle | 7 degrees | |
| **Utilities** | Voltage | 100-120/220-240 VAC | |
| | Frequency | 50/60 Hz | |
| | Current | Max: 15 A | |
| | Power | 12 KVA | |
| **Comments**: Purchase cost is $16,000.00 each | | | |

| SPECIFICATION SHEET | | | | |
|---|---|---|---|---|
| ThermoFisher® HyPerforma® 2:1 500 L Single-Use Bioreactor (Jacketed) | | | | |
| **Equipment** | ID | | PBrx-301 (+ PS-301) | |
| | Quantity | | 1 | |
| | Function | | Grow cells to higher VCD of 32.65 million cells / mL | |
| | Operation | | Perfusion, 5 days of growth | |
| **Materials** | | | In | Out |
| | Feed (with Sf-9 Cells) | | 50 L | 500 L |
| | Media (Sf-900™ II SFM) | | 2640.4 L | 2200 L |
| | Air | | 229.6 kg (25 LPM) | 229.6 kg (25 LPM) |
| | Oxygen (50%) | | 82.4 kg (8 LPM) | 74.4 kg (7.24 LPM) |
| | Carbon Dioxide | | - | 23.6 kg (1.67 LPM) |
| | Glucose | | 30.5 kg (0.254 kg/hr) | - |
| | Glutamine | | 11.3 kg (0.0943 kg/hr) | - |
| | Base (1M NaOH) | | 1.5 L | - |
| | Antifoam | | 8.1 L | - |
| **Design Data** | Material | | Stainless Steel | |
| | Temperature | | 27.5 ± 0.5 ˚C | |
| | pH | | 6.3 ± 0.1 | |
| | Pressure | | 1.04 bar | |
| | Sparger | | Frit + Drilled Hole (Dual-Sparger Configuration) | |
| | Bioreactor Geometry | Diameter | 75.6 cm | |
| | | Height | 152.4 cm (Liquid Height: 113.4 cm) | |
| | | Volume | 660 L (Working Volume: 500 L) | |
| | Impellor | Type | Pitched Blade (Quantity: 1 / # of Blades: 3) | |
| | | Diameter | 25.1 cm | |
| | | Power Num. | 2.1 | |
| | Agitation | Mixing Rate | 101 RPM | |
| | | Tip Speed | 137.2 cm/s | |
| **Utilities** | Agitation Rating | | 20 W/$m^3$ | |
| | Heating/Cooling Load | | 5,000 W | |
| | Motor Power Rating | | 372.8 W (AC), 400 W (DC) | |
| **Comments**: Purchase cost of bioreactor is $91,473.39 / perfusion system is $18,560.00 | | | | |

| SPECIFICATION SHEET | | | | |
|---|---|---|---|---|
| ThermoFisher® HyPerforma® 2:1 2000 L Single-Use Bioreactor (Jacketed) | | | | |
| **Equipment** | ID | | Brx-301 | |
| | Quantity | | 1 | |
| | Function | | Produce proteins at VCD of 8.15 million cells / mL | |
| | Operation | | Batch, 5 days of production | |
| **Materials** | | | In | Out |
| | Protein of Interest | | - | 3.75 kg |
| | Feed (with Sf-9 Cells + Baculovirus) | | 500 L (Sf-9 Cells)<br>20 L (Baculovirus) | 2000 L |
| | Media (Sf-900™ II SFM) | | 1472.9 L | - |
| | Air | | 1377.6 kg (150 LPM) | 1377.6 kg (150 LPM) |
| | Oxygen (100%) | | 61.8 kg (6 LPM) | 55.8 kg (5.43 LPM) |
| | Carbon Dioxide | | - | 17.7 kg (1.25 LPM) |
| | Glucose | | 21.6 kg (0.180 kg/hr) | - |
| | Glutamine | | 5.15 kg (0.0429 kg/hr) | - |
| | Base (1M NaOH) | | 1.1 L | - |
| | Antifoam | | 6 L | - |
| **Design Data** | Material | | Stainless Steel | |
| | Temperature | | 26.0 ± 1.0 ˚C | |
| | pH | | 6.3 ± 0.1 | |
| | Pressure | | 1.04 bar | |
| | Sparger | | Frit + Drilled Hole (Dual-Sparger Configuration) | |
| | Bioreactor Geometry | Diameter | 119.4 cm | |
| | | Height | 229.9 cm (Liquid Height: 178.7 cm) | |
| | | Volume | 2575 L (Working Volume: 2000 L) | |
| | Impeller | Type | Pitched Blade (Quantity: 1 / # of Blades: 3) | |
| | | Diameter | 39.8 cm | |
| | | Power Num. | 2.1 | |
| | Agitation | Mixing Rate | 75 RPM | |
| | | Tip Speed | 154.9 cm/s | |
| **Utilities** | Agitation Rating | | 20 W/$m^3$ | |
| | Heating/Cooling Load | | 18,000 W | |
| | Motor Power Rating | | 372.8 W (AC), 400 W (DC) | |
| **Comments**: Purchase cost is $214,065.99 | | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Grainger - Stationary Air Compressor, 2.0 (for WAVE Bioreactor 20/50EH) | | | |
| **Equipment** | ID | AC-201 | |
| | Quantity | 1 | |
| | Function | Supply air for two 20 L, working-volume, wave bioreactors | |
| | Operation | 5 days | |
| **Materials** | Air | Inlet | Outlet |
| | Flow Rate | 0.4 LPM (Total: 0.8 LPM) | 0.36 LPM (Total: 0.72 LPM) |
| | Pressure | 1.01 bar | 1.11 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Tank Size | 7.6 L | |
| | Dimensions (LxWxH) | 0.46 x 0.20 x 0.43 m | |
| | Power Requirement | 0.000167 hp | |
| **Utilities** | Maximum Power | 0.17 hp | |
| | Voltage | 120 VAC | |
| | Current | 4.3 A | |
| **Comments**: Purchase cost is $1212.50 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Grainger - Stationary Air Compressor, 2.0 (for WAVE Bioreactor 200EH) | | | |
| **Equipment** | ID | AC-101 | |
| | Quantity | 1 | |
| | Function | Supply air for three 50 L, working-volume, wave bioreactors | |
| | Operation | 3 days | |
| **Materials** | Air | Inlet | Outlet |
| | Flow Rate | 2 LPM (Total: 6 LPM) | 1.67 LPM (Total: 5.01 LPM) |
| | Pressure | 1.01 bar | 1.21 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Tank Size | 7.6 L | |
| | Dimensions (LxWxH) | 0.46 x 0.20 x 0.43 m | |
| | Power Requirement | 0.00247 hp | |
| **Utilities** | Maximum Power | 0.17 hp | |
| | Voltage | 120 VAC | |
| | Current | 4.3 A | |
| **Comments**: Purchase cost is $1212.50 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Grainger - Stationary Air Compressor, 2.0 (for 500 L Perfusion Bioreactor) | | | |
| **Equipment** | ID | AC-301 | |
| | Quantity | 1 | |
| | Function | Supply air for 500 L perfusion bioreactor | |
| | Operation | 5 days | |
| **Materials** | Air | Inlet | Outlet |
| | Flow Rate | 25 LPM | 11.69 LPM |
| | Pressure | 1.01 bar | 2.16 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Tank Size | 7.6 L | |
| | Dimensions (LxWxH) | 0.46 x 0.20 x 0.43 m | |
| | Power Requirement | 0.0478 hp | |
| **Utilities** | Maximum Power | 0.17 hp | |
| | Voltage | 120 VAC | |
| | Current | 4.3 A | |
| **Comments**: Purchase cost is $1212.50 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Grainger-Fire Sprinkler Air Compressor (for 2000 L Production Bioreactor) | | | |
| **Equipment** | ID | AC-302 | |
| | Quantity | 1 | |
| | Function | Supply air for 2000 L production bioreactor | |
| | Operation | 5 days | |
| **Materials** | Air | Inlet | Outlet |
| | Flow Rate | 150 LPM | 67.94 LPM |
| | Pressure | 1.01 bar | 2.23 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Tank Size | 114 L | |
| | Dimensions (LxWxH) | 0.46 x 0.20 x 0.43 m | |
| | Power Requirement | 0.301 hp | |
| **Utilities** | Maximum Power | 0.5 hp | |
| | Voltage | 120 VAC | |
| | Current | 9.4 A | |
| **Comments**: Purchase cost is $1095.92 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Millipore® Process-Scale Pod (2-Rack Holders) | | | |
| **Equipment** | ID | DeF-401 | |
| | Quantity | 2 | |
| | Function | Filter out large cell debris, bacteria, and some viruses by using two-stage depth filter train | |
| | Operation | 2 hours | |
| **Materials** | | In | Out |
| | Protein of Interest | 3.75 kg | 3.38 kg |
| | Feed | 2000 L | 2366 L |
| | Buffer (Purified Water) | 917 L (Wetting + Flushing) | 344 L (+ Hold-up: 207 L) |
| **Design Data** | Material | 316L Stainless Steel | |
| | Temperature | 25.0 ± 1.0 ˚C | |
| | Pressure Drop | 2.1 bar | |
| | Process Flux | 100 LMH | |
| | Flow Rate | 2002 L/hr | |
| | Filters | D0SP | X0SP |
| | Filter Material | Nonwoven, Silica Filter Aid / Polyacrylic Fiber Pulp | Silica Filter Aid / Polyacrylic Fiber Pulp |
| | Filter Quantity | 26 | 13 |
| | Capacity | 100 $L/m^2$ | 150 $L/m^2$ |
| | Pore Size | 10 µm | 0.1 µm |
| | Effective Surface Area | 0.77 $m^2$ each | 1.1 $m^2$ each |
| | Hold-up Volume | 12.9 L each<br>6.30 L each (after gravity drain)<br>5.04 L each (after blow down) | 9.47 L each<br>6.72 L each (after gravity drain)<br>5.85 L each (after blow down) |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost is $52,090.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Cytiva® Chromaflow® Chromatography Column | | | |
| **Equipment** | ID | HC-401 | |
| | Quantity | 1 | |
| | Function | Separates out recombinant protein from solution by using his-tag based resins | |
| | Operation | 43 hours | |
| **Materials** | | In | Out |
| | Protein of Interest | 3.38 kg | 3.04 kg |
| | Feed | 2366 L (473 L/run) | 1257 L |
| | Waste | - | 2366 L |
| | Equilibration Buffer | 1257 L (251 L/run) | 1257 L (251 L/run) |
| | Wash Buffer | 2513 L (503 L/run) | 2513 L (503 L/run) |
| | Elution Buffer | 1257 L (251 L/run) | - |
| | Regeneration Buffer | 1257 L (251 L/run) | 1257 L (251 L/run) |
| | Regeneration Water Wash | 1257 L (251 L/run) | 1257 L (251 L/run) |
| **Design Data** | Material | Polypropylene, 316L, S32205 Stainless Steel | |
| | Temperature | 25.0 ± 1.0 ˚C | |
| | Pressure Drop | Min: 0.99 bar<br>Max: 1.98 bar | |
| | Diameter | 40 cm | |
| | Column Height | 40 cm | |
| | Packed Bed Height | 27.5 cm | |
| | Overall Height | 158.6 cm | |
| | Volume | 50.3 L | |
| | Packed Bed Volume | 33.8 L | |
| | Total Resin Volume | 168.8 L | |
| | Binding Capacity | 20 mg/mL | |
| | Flow Rate | 150 cm/hr | |
| | # of Runs | 5 | |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost is $76,000.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| ThermoFisher® HyPerforma® 5:1 2000L Single-Use Mixer (Non-Jacketed) | | | |
| **Equipment** | ID | VI-401 | |
| | Quantity | 1 | |
| | Function | Perform viral inactivation, followed by sterile membrane filtration | |
| | Operation | 4 hours | |
| **Materials** | | In | Out |
| | Protein of Interest | 3.04 kg | 2.98 kg |
| | Feed | 1257 L | 1354 L (Feed + Acid + Base) |
| | 1M Acetic Acid | 31.4 L (30 L/hr) | - |
| | 1M Tris Base | 65.3 L (60 L/hr) | - |
| **Design Data** | Material | AISI 304 (Stainless Steel) | |
| | Temperature | 25.0 ± 1.0 ˚C | |
| | Pressure | 1.04 bar | |
| | Diameter | 135 cm | |
| | Height | 185 cm | |
| | Volume | 1353 L | |
| | Impeller Diameter | 25.02 cm | |
| | Agitation Speed | 100 - 200 RPM | |
| | Tip Speed | 39.4 - 459.7 cm/s | |
| **Utilities** | Motor Power Rating | 745.7 W | |
| | Motor Torque Rating | 18 N-m | |
| | Electrical Power Rating | 100-120 VAC, 50/60 Hz, Single, 15 A<br>220-240 VAC, 50/60 Hz, Single, 10.4 A | |
| **Comments**: Purchase cost is $160,549.49 (sterile membrane filter is $970.00) | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Millipore® QuikScale® Biochromatography Column | | | |
| **Equipment** | ID | IEX-401 | |
| | Quantity | 1 | |
| | Function | Separates out recombinant protein from solution by using charge differences in different pH's | |
| | Operation | 7 hours | |
| **Materials** | | In | Out |
| | Protein of Interest | 2.98 kg | 2.68 kg |
| | Feed | 1353 L | 370 L |
| | Waste | - | 1353 L |
| | Equilibration Buffer | 265 L | 265 L |
| | Elution Buffer | 370 L | - |
| | Equilibration Wash Buffer | 159 L | 159 L |
| | 1 M NaCl Wash Buffer | 265 L | 265 L |
| | Ultrapure Water | 529 L | 529 L |
| | 1 M Acetic Acid Wash Buffer | 265 L | 265 L |
| **Design Data** | Material | Stainless Steel 1.4539 | |
| | Temperature | 25.0 ± 1.0 ˚C | |
| | Pressure Drop | 2.4 bar | |
| | Diameter | 35 cm | |
| | Height | 55 cm | |
| | Packed Bed Height | 25 cm | |
| | Volume | 53 L | |
| | Packed Bed Volume | 21.3 L | |
| | Total Resin Volume | 21.3 L | |
| | Binding Capacity | 140 mg/mL | |
| | Flow Rate | 300 cm/hr | |
| | # of Runs | 1 | |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost is $43,320.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Millipore® Viresolve® Pre-filter + Pro Device | | | |
| **Equipment** | ID | VF-401 | |
| | Quantity | 1 | |
| | Function | Filter out viruses to satisfy final log reduction value (LRV) of 6 for viral clearance | |
| | Operation | 3 hours | |
| **Materials** | | In | Out |
| | Protein of Interest | 2.68 kg | 2.63 kg |
| | Feed | 370 L | 396 L |
| | Buffer (Wetting: Purified Water / Flushing: 50 mM Acetate) | 52 L | 26 L |
| **Design Data** | Temperature | 25.0 ± 1.0 ˚C | |
| | Pressure Drop | 2.1 bar | |
| | Dimensions (L × W × H) | 34.29 × 20.96 × 4.42 cm | |
| | Process Flux | 304.5 LMH | |
| | Flow Rate | 155.3 L/hr | |
| | Filter | Adsorptive depth prefilter | Magnus 2.1 |
| | Filter Quantity | 1 | 1 |
| | Filter Material | Diatomaceous Earth (DE) + Cellulose Fibers + Cationic Imine Groups | Membrane: PES Housing: PVDF Gaskets: Silicone |
| | Mass Throughput | 8.4 $kg/m^2$ | |
| | Effective Surface Area | 1.1 $m^2$ (Prefilter) + 0.51 $m^2$ (Magnus 2.1) | |
| | Wetting / Flushing Capacity | 50 $L/m^2$ | |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost of Viresolve® Prefilter is $1,340.00 / Viresolve® Magnus 2.1 is $10,270.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Millipore® Mobius® Smart Flexware® Assemblies for TFF, Model TF3S, Jacketed | | | |
| **Equipment** | ID | DiF-401 | |
| | Quantity | 1 | |
| | Function | Diafiltration to remove small solutes in solution and retain spike protein at a final drug concentration of 6.5 g/L | |
| | Operation | Batch, 3.5 hours for 2 runs, totaling 7 hours processing time | |
| **Materials** | | In | Out |
| | Protein of Interest | 2.63 kg | 2.60 kg |
| | Feed | 396 L | 402 L |
| | 0.1N NaOH | 68 L | 68 L |
| | 150mM NaCl / 2.5mM $NaPO_4$ | 2048 L | 2042 L |
| | Bulk Medium | 4 L/min Feed<br>(200 L Feed) | 4 L/min Retentate<br>4.7 L/min Permeate |
| **Design Data** | Temperature | 25.0 ± 1.0°C | |
| | Pressure | Feed bag: atmospheric<br>Feed and Retentate pump: 4 bar<br>Transmembrane and Permeate: 2 bar | |
| | Dimensions (L × W × H) | 2022 × 2160 × 1014 mm | |
| | Feed Container | LLDPE (linear low-density polyethylene) with jacket | |
| | Feed Volume | 200 L | |
| | Recirculation Volume | 2.2 L | |
| | Membrane Hold-up Volume | 0.7 L | |
| | Unrecoverable Volume | Virtually zero using a displacement by buffer | |
| | Membrane Area | 3.42 $m^2$ total using three (3) cassettes, each 1.14 $m^2$ | |
| **Utilities** | SmartCart | 1 kW | |
| | Tank Cart | 0.4 kW | |
| | Pump Cart | 3 kW | |

**Comments**: Purchase cost is $300,000.00
The system will be cleaned with 0.1N NaOH, then equilibrated with diafiltration buffer before running the process. There will be 2 runs, each with 200 L of feed.
The diafiltration buffer will be 150 mM NaCl, 2.5 mM NaPO4, pH 6.8–7.2 in WFI (water for injection).
Three filters of Pellicon® 3 Cassettes with Ultracel® 30kDa Membrane, D screen, 1.14 $m^2$ will be used.

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-301 | |
| | Quantity | 1 | |
| | Function | Transport liquid from WAVE bioreactor 200EH to perfusion bioreactor | |
| | Operation | 30 mins | |
| **Operations** | | In | Out |
| | Feed | 50 L (1.67 LPM) | 50 L (1.67 LPM) |
| | Pressure | 1.21 bar | 1.36 bar |
| | Temperature | 27.5˚C | 27.5˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.00056 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-302 | |
| | Quantity | 1 | |
| | Function | Transport liquid from perfusion bioreactor to production bioreactor | |
| | Operation | 30 mins | |
| **Operations** | | In | Out |
| | Feed | 500 L (16.67 LPM) | 500 L (16.67 LPM) |
| | Pressure | 1.04 bar | 1.54 bar |
| | Temperature | 27.5˚C | 27.5˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.0084 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-303 | |
| | Quantity | 1 | |
| | Function | Transport liquid from WAVE bioreactor 20/50EH to production bioreactor | |
| | Operation | 15 mins | |
| **Operations** | | In | Out |
| | Feed | 20 L (1.33 LPM) | 20 L (1.33 LPM) |
| | Pressure | 1.11 bar | 1.34 bar |
| | Temperature | 27.5˚C | 27.5˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.00067 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-401 | |
| | Quantity | 1 | |
| | Function | Transport liquid from production bioreactor through depth filtration to 3000 L storage tank | |
| | Operation | 2 hours | |
| **Operations** | | In | Out |
| | Feed | 2000 L (33.37 LPM) | 2000 L (33.37 LPM) |
| | Pressure | 1.04 bar | 3.14 bar |
| | Temperature | 26.0˚C | 26.0˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.021 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-402 | |
| | Quantity | 1 | |
| | Function | Transport liquid from 3000 L storage tank through his-tag chromatography column to 2000 L single-use mixer | |
| | Operation | 2 days | |
| **Operations** | | In | Out |
| | Feed | 2366 L (3.13 LPM) | 2366 L (3.13 LPM) |
| | Pressure | 1.04 bar | 2.03 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.0013 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-403 | |
| | Quantity | 1 | |
| | Function | Transport liquid from 2000 L single-use mixer through IEX chromatography column to 500 L storage tank 1 | |
| | Operation | 7 hours | |
| **Operations** | | In | Out |
| | Feed | 1353 L (4.82 LPM) | 1353 L (4.82 LPM) |
| | Pressure | 1.04 bar | 3.44 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.0013 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-404 | |
| | Quantity | 1 | |
| | Function | Transport liquid from 500 L storage tank 1 through viral filtration to 500 L storage tank 2 | |
| | Operation | 3 hours | |
| **Operations** | | In | Out |
| | Feed | 370.4 L (2.59 LPM) | 370.4 L (2.59 LPM) |
| | Pressure | 1.04 bar | 3.14 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.00072 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-405 | |
| | Quantity | 1 | |
| | Function | Transport liquid from 500 L Storage Tank 2 to diafiltration feed tank | |
| | Operation | 30 mins | |
| **Operations** | | In | Out |
| | Feed | 395.9 L (13.33 LPM) | 395.9 L (13.33 LPM) |
| | Pressure | 1.04 bar | 1.28 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.0030 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® Digital Drive, Modular, Washdown Pump | | | |
| **Equipment** | ID | PP-406 | |
| | Quantity | 1 | |
| | Function | Transport liquid from diafiltration feed tank to 500 L Storage Tank 3 | |
| | Operation | 30 mins | |
| **Operations** | | In | Out |
| | Feed | 401.7 L (13.33 LPM) | 401.7 L (13.33 LPM) |
| | Pressure | 1.01 bar | 1.09 bar |
| | Temperature | 25.0˚C | 25.0˚C |
| **Design Data** | Control Type | Digital Variable Speed | |
| | Washdown Capable | Yes | |
| | Flow Rate | Min: 1.3 LPM<br>Max: 42 LPM | |
| | Controller Dimensions (LxWxH) | 0.28 x 0.11 x 0.23 m | |
| | Drive Dimensions (LxWxH) | 0.40 x 0.38 x 0.69 m | |
| | Power Requirement | 0.0037 hp | |
| **Utilities** | Maximum Power | 0.75 hp | |
| | Voltage | 115 VAC (90 to 130) | |
| | Current | 5.7 A | |
| | Frequency | 50/60 Hz | |
| **Comments**: Purchase cost is $7390.00 | | | |

| SPECIFICATION SHEET | | | |
|---|---|---|---|
| Masterflex B/T® PerfectPosition™ Pump Tubing | | | |
| **Equipment** | ID | N/A | |
| | Quantity | 90 (B/T 87) + 10 (B/T 91) | |
| | Function | Transport of fluid between process units | |
| | Operation | Continuous | |
| **B/T 87** | Length | 10 ft (3 m) | |
| | Inner Diameter | 0.5 in (12.7 mm) | |
| | Material | Platinum-Cured Silicone | |
| | Sterilization | Autoclave | |
| | | Min | Max |
| | Flow Rate | 0.67 LPM | 18.9 LPM |
| | Temperature (Dynamic) | -40°C | 100°C |
| | Temperature | -50°C | 230°C |
| | Pressure | N/A | 2.4 bar |
| **B/T 91** | Length | 10 ft (3 m) | |
| | Inner Diameter | 0.75 in (19 mm) | |
| | Material | Platinum-Cured Silicone | |
| | Sterilization | Autoclave | |
| | | Min | Max |
| | Flow Rate | 1.4 LPM | 42 LPM |
| | Temperature (Dynamic) | -40°C | 100°C |
| | Temperature | -50°C | 230°C |
| | Pressure | N/A | 2.06 bar |
| **Utilities** | N/A | | |
| **Comments**: Purchase cost of B/T 87 is $484.00 per pack of 10 / B/T 91 is $691.00 per pack of 10<br>B/T tubing goes with the B/T peristaltic pumps. The products are available as B/T 87 and B/T 91 tubes. | | | |

# 17. Equipment Cost Summary

*Table 17.1* lists the purchase and bare module costs of all the required equipment for this process. While we will not actually buy the equipment, we are assuming these units or similar pieces are available at the CMO. By calculating the total bare module cost, we can estimate the CMO costs and subsequently estimate a reasonable monthly fee to use their equipment. The bare module costs were calculated using the purchase costs obtained from vendors and the bare module factors from Table 16.11 in Seider et al. (2017) [93]. For equipment not included in Table 16.11, bare module factors were estimated. Specifically, for storage tanks, bare module factor of 4.0 was assumed in the equipment costing spreadsheet. A factor of 4.16 was assumed for mixers and bioreactors, which was considered to be similar in function to a fermenter. Other pieces of equipment without a bare module factor were assigned the default value of 3.21 in the spreadsheet. A CEI index of 600 for 2021 was used. The single-use mixer cost was assumed to be ¾ the price of the bioreactor cost. Finally, the biological and chemical lab was assumed to be 5% of the total capital and operating costs.

*Table 17.1.* Purchase and Bare Module Costs of Equipment

| Equipment | ID | Purchase Cost | Quantity | Bare Module Factor | Bare Module Cost |
|---|---|---|---|---|---|
| **Raw Material Storage** | | | | | |
| 200 L Media Vessel | M-101 | $ 4,000.00 | 1 | 4.0 | $ 16,000.00 |
| 2000 L Media Vessel | M-302 | $ 10,210.53 | 1 | 4.0 | $ 40,842.12 |
| 3000 L Media Vessel | M-301 | $ 12,421.05 | 1 | 4.0 | $ 49,684.20 |
| 100 L Glucose Vessel | GC-301 | $ 2,000.00 | 1 | 4.0 | $ 8,000.00 |
| 500 L Glutamine Vessel | GT-301 | $ 3,000.00 | 1 | 4.0 | $ 12,000.00 |
| 20 L Antifoam Vessel | AF-301 | $ 269.90 | 1 | 4.0 | $ 1,079.60 |
| 500 L Storage Tank | ST-402<br>ST-403<br>ST-404 | $ 1,500.00 | 3 | 4.0 | $ 18,000.00 |
| 3000 L Storage Tank | ST-401 | $ 6,000.00 | 1 | 4.0 | $ 24,000.00 |

| Upstream Equipment | | | | | |
|---|---|---|---|---|---|
| WAVE™ Bioreactor System 20/50EH | W-201a<br>W-201b | $ 4,000.00 | 2 | 3.21 | $ 25,680.00 |
| WAVE™ Bioreactor System 200EH | W-101a<br>W-101b<br>W-101c | $ 16,000.00 | 3 | 3.21 | $ 154,080.00 |
| 500 L Bioreactor (Jacketed) | PBrx-301 | $ 91,473.39 | 1 | 4.16 | $ 380,529.30 |
| 500 L Perfusion System | PS-301 | $ 18,560.00 | 1 | 3.21 | $ 59,577.60 |
| 2000 L Bioreactor (Jacketed) | Brx-301 | $ 214,065.99 | 1 | 4.16 | $ 890,514.53 |
| **Downstream Equipment** | | | | | |
| 2-Rack Process Pod (Filter Frame) | DeF-401 | $ 52,090.00 | 1 | 2.32 | $ 120,848.80 |
| His-tag Chromatography Column | HC-401 | $ 76,000.00 | 1 | 4.16 | $ 316,160.00 |
| 2000 L Single-Use Mixer (Non-Jacketed) | VI-401 | $ 160,549.49 | 1 | 4.16 | $ 667,885.90 |
| Ion Exchange Chromatography Column | IEX-401 | $ 43,320.00 | 1 | 4.16 | $ 180,211.20 |
| Diafiltration Assembly (Jacketed) | DiF-401 | $ 300,000.00 | 1 | 3.21 | $ 963,000.00 |
| **Transport Equipment** | | | | | |
| Air Compressor with Tank (for WAVE Bioreactor 20/50EH) | AC-201 | $ 1,212.50 | 1 | 2.15 | $ 2,606.88 |
| Air Compressor with Tank (for WAVE Bioreactor 200EH) | AC-101 | $ 1,212.50 | 1 | 2.15 | $ 2,606.88 |
| Air Compressor with Tank (for 500 L Perfusion Bioreactor) | AC-301 | $ 1,212.50 | 1 | 2.15 | $ 2,606.88 |
| Air Compressor with Tank (for 2000 L Production Bioreactor) | AC-302 | $ 1,095.92 | 1 | 2.15 | $ 2,356.23 |
| Peristaltic Pump | PP-301<br>PP-302<br>PP-303<br>PP-401<br>PP-402<br>PP-403<br>PP-404<br>PP-405 | $ 7,390.00 | 9 | 3.30 | $ 219,483.00 |

| | | | | | |
|---|---|---|---|---|---|
| | PP-406 | | | | |
| **Miscellaneous** | | | | | |
| Biological/Chemical Lab | - | $ 270,622.93 | 1 | - | $ 270,622.93 |
| | | | | | |
| | | | | Total | $ 4,428,376.03 |

The most significant investments include the diafiltration unit, 2000 L bioreactor, and 2000 L single-use mixer. This is expected, as these are the largest or most complex pieces of equipment. Unfortunately, they are completely necessary and cannot be substituted for cheaper solutions. Additionally, they should be able to be used for many years, as we are using single-use bags that should preserve the equipment quality. The other bioreactors and chromatography columns also account for a large portion of the investment costs. Finally, we are purchasing storage tanks, drums, and vessels which accumulate to be a large portion of our capital investment.

# 18. Fixed Capital Investment Summary

The company will partner with a CMO to manufacture the vaccine for emergency use. Based on consultation with Professor Bruce Vrana, economics were analyzed using two spreadsheets made from the Profitability 4.0 Excel spreadsheet template: one for the CMO to determine our rental fee, and one for our company to price the variable costs. All fixed costs were incorporated into the CMO spreadsheet. In this costing exercise, the CMO was assumed to have invested the necessary capital in 2015 for the plant to operate for 15 years. The CMO was assumed to make 1 product a month, totaling 12 products each year.

## 18.1 Total Bare Module Cost

All of the required equipment bare module costs were input into the CMO spreadsheet to determine the fixed costs for our process. Each piece of equipment was classified as either storage or process machinery. Process machinery was equipment bought from a vendor in standard sizes. There was no fabricated equipment, as none of the units required custom design. The biochemical lab was considered to be a part of other equipment. The total bare module costs for our process are listed in *Figure 18.1* below.

| **Total Bare Module Costs:** | | | |
|---|---|---|---|
| **Fabricated Equipment** | $ | - | |
| **Process Machinery** | $ | 3,988,147 | |
| **Spares** | $ | - | |
| **Storage** | $ | 169,606 | |
| **Other Equipment** | $ | 270,623 | |
| **Catalysts** | $ | - | |
| **Computers, Software, Etc.** | $ | - | |
| **Total Bare Module Costs:** | | | **$ 4,428,376** |

*Figure 18.1.* Total Bare Module Costs for the CMO facility.

The total bare module cost for fixed equipment is roughly $4.4 million. This consists primarily of the process machinery required to run this process. Spares were not accounted for, but this cost is wrapped into a multiplier used later in the estimation of what the CMO might charge the company. The storage units do not make up a significant amount of our costs, as they are not nearly as expensive as the process equipment.

## 18.2 Total Permanent Investment

Other fixed costs such as royalties and facilities were considered to calculate the total permanent investment of the CMO. No default spreadsheet values for these factors were changed. *Figure 18.2.1* below breaks down how the additional fixed costs were calculated. Guthrie's factored-cost methods were used, thus our cost of site preparation and service facilities, for example, were taken as percentages of our bare module costs [93].

| Total Permanent Investment | |
|---|---|
| Cost of Site Preparations: | **5.00% of Total Bare Module Costs** |
| Cost of Service Facilities: | **5.00% of Total Bare Module Costs** |
| Allocated Costs for utility plants and related facilities: | **$0** |
| Cost of Contingencies and Contractor Fees: | **18.00% of Direct Permanent Investment** |
| Cost of Land: | **2.00% of Total Depreciable Capital** |
| Cost of Royalties: | **$0** |
| Cost of Plant Start-Up: | **10.00% of Total Depreciable Capital** |

*Figure 18.2.1*. Total Permanent Investment inputs from Profitability 4.0 Spreadsheet

The royalties remained zero, as we do not expect royalty costs. Additionally, the costs for utility plants were not included since municipal utilities will be used instead. The total permanent investment comes out to about $6.7 million for the CMO. Professor Vrana suggested using a sight factor of 1.05 for North Carolina. Seider et al. (2017) defines an investment site factor of 1.00 for states on the Gulf Coast and 1.10 for states in the Northeast [93]. Since North Carolina lies between these regions, 1.05 was taken to be a reasonable estimate. *Figure 18.2.2* shows the overall investment summary for this project based on CMO fixed costs.

| Investment Summary | | | |
|---|---|---|---|
| **Total Bare Module Costs:** | | | |
| Fabricated Equipment | $ | - | |
| Process Machinery | $ | 3,988,147 | |
| Spares | $ | - | |
| Storage | $ | 169,606 | |
| Other Equipment | $ | 270,623 | |
| Catalysts | $ | - | |
| Computers, Software, Etc. | $ | - | |
| **Total Bare Module Costs:** | | | $ 4,428,376 |
| **Direct Permanent Investment** | | | |
| Cost of Site Preparations: | $ | 221,419 | |
| Cost of Service Facilities: | $ | 221,419 | |
| Allocated Costs for utility plants and related facilities: | $ | - | |
| **Direct Permanent Investment** | | | $ 4,871,214 |
| **Total Depreciable Capital** | | | |
| Cost of Contingencies & Contractor Fees | $ | 876,818 | |
| **Total Depreciable Capital** | | | $ 5,748,032 |
| **Total Permanent Investment** | | | |
| Cost of Land: | $ | 114,961 | |
| Cost of Royalties: | $ | - | |
| Cost of Plant Start-Up: | $ | 574,803 | |
| Total Permanent Investment - Unadjusted | | | $ 6,437,796 |
| Site Factor | | | 1.05 |
| **Total Permanent Investment** | | | $ 6,759,686 |

*Figure 18.2.2.* Total Permanent Investment Summary

## 18.3 Remaining Fixed Costs

*Figure 18.3* below summarizes the remaining fixed costs used to determine the total capital investment for the CMO. Notably, it includes operations, maintenance, overhead, property taxes, and depreciation. No spreadsheet defaults were changed other than the specification of 5 operators per shift assuming 5 shifts.

**Fixed Costs**

**Operations**

| | | |
|---|---|---|
| Operators per Shift: | **5** | **(assuming 5 shifts)** |
| Direct Wages and Benefits: | **$40** | **/operator hour** |
| Direct Salaries and Benefits: | **15%** | **of Direct Wages and Benefits** |
| Operating Supplies and Services: | **6%** | **of Direct Wages and Benefits** |
| Technical Assistance to Manufacturing: | **$60,000.00** | **per year, for each Operator per Shift** |
| Control Laboratory: | **$65,000.00** | **per year, for each Operator per Shift** |

**Maintenance**

| | | |
|---|---|---|
| Wages and Benefits: | **4.50%** | **of Total Depreciable Capital** |
| Salaries and Benefits: | **25%** | **of Maintenance Wages and Benefits** |
| Materials and Services: | **100%** | **of Maintenance Wages and Benefits** |
| Maintenance Overhead: | **5%** | **of Maintenance Wages and Benefits** |

**Operating Overhead**

| | | |
|---|---|---|
| General Plant Overhead: | **7.10%** | **of Maintenance and Operations Wages and Benefits** |
| Mechanical Department Services: | **2.40%** | **of Maintenance and Operations Wages and Benefits** |
| Employee Relations Department: | **5.90%** | **of Maintenance and Operations Wages and Benefits** |
| Business Services: | **7.40%** | **of Maintenance and Operations Wages and Benefits** |

**Property Taxes and Insurance**

| | | |
|---|---|---|
| Property Taxes and Insurance: | **2%** | **of Total Depreciable Capital** |

**Straight Line Depreciation**

| | | |
|---|---|---|
| Direct Plant: | 8.00% | **of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities** |
| Allocated Plant: | 6.00% | **of 1.18 times the Allocated Costs for Utility Plants and Related Facilities** |

**Other Annual Expenses**

| | |
|---|---|
| Rental Fees (Office and Laboratory Space): | **$0** |
| Licensing Fees: | **$0** |
| Miscellaneous: | **$0** |

**Depletion Allowance**

| | |
|---|---|
| Annual Depletion Allowance: | **$0** |

*Figure 18.3.* Remaining Fixed Costs of the CMO.

## 18.4 Total Fixed Cost Summary

*Figure 18.4* below summarizes the total fixed cost for the CMO to operate, which is estimated to be about $7 million.

| Fixed Cost Summary | | | |
|---|---|---|---|
| **Operations** | | | |
| | Direct Wages and Benefits | $ | 2,080,000 |
| | Direct Salaries and Benefits | $ | 312,000 |
| | Operating Supplies and Services | $ | 124,800 |
| | Technical Assistance to Manufacturing | $ | 1,500,000 |
| | Control Laboratory | $ | 1,625,000 |
| | **Total Operations** | **$** | **5,641,800** |
| **Maintenance** | | | |
| | Wages and Benefits | $ | 258,661 |
| | Salaries and Benefits | $ | 64,665 |
| | Materials and Services | $ | 258,661 |
| | Maintenance Overhead | $ | 12,933 |
| | **Total Maintenance** | **$** | **594,921** |
| **Operating Overhead** | | | |
| | General Plant Overhead: | $ | 192,788 |
| | Mechanical Department Services: | $ | 65,168 |
| | Employee Relations Department: | $ | 160,204 |
| | Business Services: | $ | 200,934 |
| | **Total Operating Overhead** | **$** | **619,095** |
| **Property Taxes and Insurance** | | | |
| | Property Taxes and Insurance: | $ | 114,961 |
| **Other Annual Expenses** | | | |
| | Rental Fees (Office and Laboratory Space): | $ | - |
| | Licensing Fees: | $ | - |
| | Miscellaneous: | $ | - |
| | **Total Other Annual Expenses** | **$** | **-** |
| **Total Fixed Costs** | | **$** | **6,970,776** |

*Figure 18.4.* Fixed Cost Summary for the CMO

## 18.5 Working Capital

*Figure 18.5.1* shows the CMO working capital calculation parameters for accounts receivable, cash reserves, accounts payable, vaccine inventory, and raw materials. The default

values were not changed. *Figure 18.5.2* shows the total capital investment comes out to $7.9 million for the CMO. No raw materials or variable costs are included, as these costs were assumed to be transferred to the company partnering with the CMO.

| Working Capital | | | |
|---|---|---|---|
| Accounts Receivable | ⇨ | 30 | Days |
| Cash Reserves (excluding Raw Materials) | ⇨ | 30 | Days |
| Accounts Payable | ⇨ | 30 | Days |
| Vaccine Inventory | ⇨ | 4 | Days |
| Raw Materials | ⇨ | 2 | Days |

*Figure 18.5.1.* Calculation Parameters for Working Capital for the CMO.

| Working Capital | | | | |
|---|---|---|---|---|
| | 2016 | 2017 | 2018 |
| Accounts Receivable | $ 453,699 | $ 226,849 | $ 226,849 |
| Cash Reserves | $ 229,176 | $ 114,588 | $ 114,588 |
| Accounts Payable | $ - | $ - | $ - |
| Vaccine Inventory | $ 60,493 | $ 30,247 | $ 30,247 |
| Raw Materials | $ - | $ - | $ - |
| **Total** | **$ 743,368** | **$ 371,684** | **$ 371,684** |
| *Present Value at 15%* | *$ 646,407* | *$ 281,047* | *$ 244,388* |
| **Total Capital Investment** | | **$ 7,931,527** | |

*Figure 18.5.2.* Total Working Capital Summary for the CMO.

After completing the analysis for the CMO, an estimation was needed for the fee that the CMO chould charge our company to use their space for 3 months. Two months would be used for experiments and training personnel, while the last month would be used for actual production. The plant was assumed to be operational for 15 years, and an IRR of minimally 15% was required for the CMO to be satisfied. To achieve this, the CMO needs to earn approximately $1.15 million per month assuming the facility operates at 80% design capacity. This fee was multiplied by 3 to account for the 3 month rental, then multiplied by 5 to account for current competition in the CMO market and the profit the CMO would want to incur. This number was taken to be a rental fee in our company spreadsheet, where the variable costs and other remaining costs were input and explained in Section 19 and summarized in *Figure 19.5*.

# 19. Operating Cost

## 19.1 Process Materials

*Table 19.1* lists the cost of process materials for one operation campaign, with components listed by its usage in each unit operation: storage materials, transport materials, upstream materials, and downstream materials. The plant spends about $928,000 on raw products and process materials per campaign. Each dose will take $0.0019 of process materials to manufacture, which is significantly below the selling price of $16 per dose [94].

*Table 19.1.* Cost of Process Materials per Run, Grouped by Unit Operation

| Materials | Unit Cost | Quantity | Unit | Purchase Cost |
|---|---|---|---|---|
| **Storage Materials** | | | | |
| 50 L WAVE Bioreactor Cell Culture Bags | $ 322.16/Cellbag | 2 | Cellbag | $ 644.32 |
| 200 L WAVE Bioreactor Cell Culture Bags | $ 1,288.64/Cellbag | 3 | Cellbag | $ 3,865.92 |
| 500 L BioProcess Containers | $ 4,453.99/BPC | 4 | BPC | $ 17,815.95 |
| 2000 L BioProcess Containers | $ 10,233.13/BPC | 2 | BPC | $ 20,466.26 |
| 2500 L BioProcess Containers | $ 14,058.37/BPC | 1 | BPC | $ 14,058.37 |
| **Transport Materials** | | | | |
| Sterile Filter (for Liquid) | $ 152.60/filter | 15 | filter | $ 2,289.00 |
| 10 ft Peristaltic Tube (B/T 87) | $ 48.40/ft (per 10 ft) | 900 | ft | $ 43,560.00 |
| 10 ft Peristaltic Tube (B/T 91) | $ 69.10/ft (per 10 ft) | 100 | ft | $ 6,910.00 |
| 10x Fitting, Nylon, Straight, Hose Barb Union (0.5 in) | $ 2.13/fitting (pack of 10) | 50 | fitting | $ 106.25 |
| 10x Fitting, Nylon, Straight, Hose Barb Union (0.75 in) | $ 2.65/fitting (pack of 10) | 10 | fitting | $ 26.50 |
| **Upstream Materials** | | | | |
| 125 mL Erlenmeyer Shake Flask | $ 15.70/flask | 4 | flask | $ 62.80 |

| | | | | |
|---|---|---|---|---|
| 250 mL Erlenmeyer Shake Flask | $ 17.70/flask | 2 | flask | $ 35.40 |
| 500 mL Erlenmeyer Shake Flask | $ 27.10/flask | 4 | flask | $ 108.40 |
| 5 L Erlenmeyer Shake Flask | $ 166.00/flask | 3 | flask | $ 498.00 |
| Cell Bank Inoculum | $ 450.00/vial (50M cells) | 4 | vial | $ 1,800.00 |
| Baculovirus Stock | $ 112.50/mL (per 10 mL) | 40 | mL | $ 4,500.00 |
| Sf-900 II SFM | $ 67.50/L (per 20 L) | 4300 | L | $ 290,250.00 |
| Oxygen (+ Tank) | $ 0.0677/L (per 7079 L) | 106,185 | L | $ 7,186.80 |
| Glucose | $ 10.72/kg (per 25 kg) | 75 | kg | $ 804.00 |
| Glutamine | $ 509.00/kg | 20 | kg | $ 10,180.00 |
| Base (1M NaOH) | $ 26.36/L (per 4 L) | 4 | L | $ 105.45 |
| Antifoam | $ 1,164.00/L (per 50 mL) | 14.1 | L | $ 16,412.40 |
| Purified Water / 100 L Glucose Vessel | $ 5.12/L (per 200 L) | 82.4 | L | $ 421.4884 |
| Purified Water / 100 L Glutamine Vessel | $ 5.12/L (per 200 L) | 484.3 | L | $ 2,479.6160 |
| **Downstream Materials** | | | | |
| D0SP Filter | $ 834.00/filter | 26 | filter | $ 21,684.00 |
| X0SP Filter | $ 1,050.00/filter | 13 | filter | $ 13,650.00 |
| Depth Filtration Wetting / Flushing Buffer (Purified Water) | $ 5.12/L (per 200 L) | 917 | L | $ 4,690.59 |
| His-tag Resin | $ 6,660.00/L | 34 | L | $ 226,440.00 |
| His-tag Sodium Phosphate | $ 206.44/kg | 16.1 | kg | $ 3,323.68 |
| His-tag NaCl | $ 40.26/kg | 95.6 | kg | $ 3,848.51 |
| His-tag Imidazole | $ 154.00/kg | 15.9 | kg | $ 2,448.60 |
| His-tag MES | $ 472.00/kg | 5.4 | kg | $ 2,548.80 |
| His-tag Purified Water | $ 5.12/L (per 200 L) | 7555 | L | $ 38,644.96 |
| 1M Acetic Acid | $ 26.75/L (per 20 L) | 40 | L | $ 1,070.00 |
| 1M Tris Base | $ 91.00/kg (per 5 kg) | 10 | L | $ 910.00 |
| Sterile Membrane Filter | $ 970.00/filter | 1 | filter | $ 970.00 |
| Ion-Exchange Resin | $ 3,600.00/L | 26.5 | L | $ 95,400.00 |
| Ion-Exchange NaCl for Buffers | $ 52.20/kg | 34 | kg | $ 1,774.80 |
| Ion-Exchange Wash Buffer (Purified Water) | $ 5.12/L (per 200 L) | 159 | L | $ 813.31 |
| Ion-Exchange Elution Buffer (Acetic Acid) | $ 14.24/L (per 2.5 L) | 7.5 | L | $ 106.80 |
| Ion-Exchange Elution | $ 10.20/L (per 10 L) | 30 | L | $ 306.00 |

| | | | | |
|---|---|---|---|---|
| Buffer (Sodium Acetate) | | | | |
| Ion-Exchange Elution Buffer (Purified Water) | $ 5.12/L (per 200 L) | 334 | L | $ 1,708.46 |
| Ion-Exchange Cleaning Buffer (Purified Water) | $ 5.12/L (per 200 L) | 530 | L | $ 2,711.03 |
| Ion-Exchange Cleaning Buffer (Acetic Acid) | $ 14.24/L (per 2.5 L) | 265 | L | $ 3,773.60 |
| Adsorptive Pre-Filter (Viral Pre-filter) | $ 1,340.00/filter | 1 | filter | $ 1,340.00 |
| Magnus 2.1 Pro Device (Viral Filter) | $ 10,270.00/filter | 1 | filter | $ 10,270.00 |
| Viral Filtration Wetting Buffer (Purified Water) | $ 5.12/L (per 200 L) | 26 | L | $ 132.99 |
| Viral Filtration Flushing Buffer (50 mM Acetate) | $ 210.08/L (per 250 mL) | 26 | L | $ 5,462.08 |
| Diafiltration Filter (Pellicon 3, 30kD Ultracel, D Screen) | $ 7,160.00/cassette | 3 | cassette | $ 21,480.00 |
| Diafiltration Buffer (NaCl) | $ 16.48/kg (per 25 kg) | 412 | kg | $ 6,789.76 |
| Diafiltration Buffer (NaH2PO4 · H2O) | $ 151.14/kg (per 500 g) | 0.5 | kg | $ 75.57 |
| Diafiltration Buffer (Na2HPO4 · 12H2O) | $ 184.80/kg (per 500 g) | 0.5 | kg | $ 92.40 |
| Diafiltration Buffer (Purified Water) | $ 5.12/L (per 200 L) | 2116 | L | $ 10,823.66 |
| Diafiltration Cleaning Buffer (0.1N NaOH) | $ 133.80/kg (per 500 g) | 0.5 | kg | $ 66.90 |

## 19.2 Utilities

Data from the U.S. Energy Information Administration lists the average price of electricity for the industrial sector in North Carolina as $0.0575 per kilowatt-hour in January 2021 [95]. For the treatment of solid waste, the cost for treating biowaste at a local incinerator company was calculated as ten times the cost of treating municipal waste, as per consultation with Dr. Cohen. The CMO facility is located in Durham, North Carolina, where the cost of treating municipal waste is $47.50 per ton [96][97], which we estimated would cost $475 to treat

one ton of biowaste. Our utilities costs are summarized below in *Table 19.2*. The most significant costs include the electricity for the media vessels and wastewater treatment. In total, the utilities cost about $6,000 per run.

*Table 19.2.* Cost of Utilities By Unit Operation

| Utilities | Unit Cost | Amount | Unit | Utilities Cost |
|---|---|---|---|---|
| **Utilities per Process Unit** | | | | |
| Chilled Water / 500 L Perfusion Bioreactor | $ 0.0178/kWh | 79.44 | kWh | $ 1.41 |
| Chilled Water / 2000 L Bioreactor | $ 0.0178/kWh | 83.04 | kWh | $ 1.48 |
| Chilled Water / Diafiltration Assembly | $ 0.0178/kWh | 23.80 | kWh | $ 0.42 |
| Electricity / WAVE™ Bioreactor System 20/50EH | $ 0.0575/kWh | 82.80 | kWh | $ 4.76 |
| Electricity / WAVE™ Bioreactor System 200EH | $ 0.0575/kWh | 259.20 | kWh | $ 14.90 |
| Electricity / 500 L Perfusion Bioreactor | $ 0.0575/kWh | 288.00 | kWh | $ 16.56 |
| Electricity / 2000 L Bioreactor | $ 0.0575/kWh | 288.00 | kWh | $ 16.56 |
| Electricity / 2000 L Single-Use Mixer | $ 0.0575/kWh | 10.00 | kWh | $ 0.5750 |
| Electricity / Diafiltration Assembly | $ 0.0575/kWh | 30.80 | kWh | $ 1.7710 |
| Electricity / Air Compressor (for WAVE Brx 20/50EH) | $ 0.0575/kWh | 15.30 | kWh | $ 0.8798 |
| Electricity / Air Compressor (for WAVE Brx 200EH) | $ 0.0575/kWh | 9.18 | kWh | $ 0.5279 |
| Electricity / Air Compressor (for 500 L Perfusion Brx) | $ 0.0575/kWh | 15.30 | kWh | $ 0.8798 |
| Electricity / Air Compressor (for 2000 L Production Brx) | $ 0.0575/kWh | 45.00 | kWh | $ 2.5875 |
| Electricity / Upstream Peristaltic Pump (BV WAVE to N Brx) | $ 0.0575/kWh | 0.14 | kWh | $ 0.0081 |
| Electricity / Upstream Peristaltic Pump (Sf-9 WAVE to N-1 Brx) | $ 0.0575/kWh | 0.28 | kWh | $ 0.0162 |
| Electricity / Upstream Peristaltic Pump (N-1 Brx to N Brx) | $ 0.0575/kWh | 0.28 | kWh | $ 0.0162 |
| Electricity / Downstream | $ 0.0575/kWh | 1.13 | kWh | $ 0.0647 |

| | | | | |
|---|---|---|---|---|
| Peristaltic Pump (for Depth Filtration) | | | | |
| Electricity / Downstream Peristaltic Pump (for His-tag Column) | $ 0.0575/kWh | 27.00 | kWh | $ 1.5525 |
| Electricity / Downstream Peristaltic Pump (for IEX Column) | $ 0.0575/kWh | 3.94 | kWh | $ 0.2264 |
| Electricity / Downstream Peristaltic Pump (for Viral Filtration) | $ 0.0575/kWh | 1.69 | kWh | $ 0.0970 |
| Electricity / Downstream Peristaltic Pump (for Diafiltration in) | $ 0.0575/kWh | 0.28 | kWh | $ 0.0162 |
| Electricity / Downstream Peristaltic Pump (for Diafiltration out) | $ 0.0575/kWh | 0.28 | kWh | $ 0.0162 |
| **Utilities per Storage Unit** | | | | |
| Electricity / 200 L Media Vessel | $ 0.0575/kWh | 900 | kWh | $ 51.7500 |
| Electricity / 2000 L Media Vessel | $ 0.0575/kWh | 1500 | kWh | $ 86.2500 |
| Electricity / 3000 L Media Vessel | $ 0.0575/kWh | 1500 | kWh | $ 86.2500 |
| Electricity / 100 L Glucose Vessel | $ 0.0575/kWh | 180 | kWh | $ 10.3500 |
| Electricity / 500 L Glutamine Vessel | $ 0.0575/kWh | 360 | kWh | $ 20.7000 |
| **Total Utilities** | | | | |
| Chilled Water | $ 0.0178/kWh | 186.28 | kWh | $ 3.31 |
| Electricity | $ 0.0575/kWh | 5435.80 | kWh | $ 317.32 |
| **Waste Treatment** | | | | |
| Solid Waste Treatment | $ 0.24/lb | 50 | lb | $ 11.88 |
| Wastewater Treatment | $ 0.33/kg | 16453.54 | kg | $ 5429.67 |

## 19.3 Labor Costs

Our plant will require 5 operators and 1 engineering manager per shift. There will be a total of 5 shifts per day at 8 hours each for continuous operation of 24/7, including weekends. Operators will receive a salary of $60,000, and the manager will receive a salary of $75,000. The

costs for the 5 operators per shift were included in the CMO analysis in Section 18. The cost for our own engineering manager, who will oversee plant operations and quality control to ensure adherence to GMP standards, was included in our company spreadsheet.

## 19.4 Variable Costs

Other variable costs for the company include selling and transfer expenses, research, administrative expenses, and management incentive compensation. These costs were included in addition the monthly fee for the CMO in the company spreadsheet. Both the company and the CMO were assumed to bear research and administrative expenses. *Figure 19.4.1* shows the parameters used.

| Variable Costs | |
|---|---|
| **General Expenses:** | |
| Selling / Transfer Expenses: | **3.00% of Sales** |
| Direct Research: | **4.80% of Sales** |
| Allocated Research: | **0.50% of Sales** |
| Administrative Expense: | **2.00% of Sales** |
| Management Incentive Compensation: | **1.25% of Sales** |

*Figure 19.4.1.* Calculation Parameters for Variable Costs

*Figure 19.4.2* summarizes the variable costs from our company spreadsheet to account for raw materials, utilities and general expenses. The total variable cost is $925 million.

| Variable Cost Summary | | | |
|---|---|---|---|
| **Variable Costs at 100% Capacity:** | | | |
| **General Expenses** | | | |
| Selling / Transfer Expenses: | | $ | 240,000,000 |
| Direct Research: | | $ | 384,000,000 |
| Allocated Research: | | $ | 40,000,000 |
| Administrative Expense: | | $ | 160,000,000 |
| Management Incentive Compensation: | | $ | 100,000,000 |
| **Total General Expenses** | | $ | 924,000,000 |
| **Raw Materials** | $0.001856 per dose of Vaccine | | $927,943 |
| **Byproducts** | $0.000000 per dose of Vaccine | | $0 |
| **Utilities** | $0.000001 per dose of Vaccine | | $328 |
| **Total Variable Costs** | | **$** | **924,928,272** |

*Figure 19.4.2.* Variable Cost Summary for the Company

## 19.5 Remaining Fixed Costs

The fixed costs for our company include the CMO rental fee and minor costs for operation and overhead. *Figure 19.5* summarizes the fixed costs, which total $18.8 million.

**Fixed Cost Summary**

| | | | |
|---|---|---|---|
| **Operations** | | | |
| | Direct Wages and Benefits | $ | 1,040,000 |
| | Direct Salaries and Benefits | $ | 156,000 |
| | Operating Supplies and Services | $ | 62,400 |
| | Technical Assistance to Manufacturing | $ | - |
| | Control Laboratory | $ | - |
| | **Total Operations** | **$** | **1,258,400** |
| **Maintenance** | | | |
| | Wages and Benefits | $ | - |
| | Salaries and Benefits | $ | - |
| | Materials and Services | $ | - |
| | Maintenance Overhead | $ | - |
| | **Total Maintenance** | **$** | **-** |
| **Operating Overhead** | | | |
| | General Plant Overhead: | $ | 84,916 |
| | Mechanical Department Services: | $ | 28,704 |
| | Employee Relations Department: | $ | 70,564 |
| | Business Services: | $ | 88,504 |
| | **Total Operating Overhead** | **$** | **272,688** |
| **Property Taxes and Insurance** | | | |
| | Property Taxes and Insurance: | $ | - |
| **Other Annual Expenses** | | | |
| | Rental Fees (Office and Laboratory Space): | $ | 17,250,000 |
| | Licensing Fees: | $ | - |
| | Miscellaneous: | $ | - |
| | **Total Other Annual Expenses** | **$** | **17,250,000** |
| **Total Fixed Costs** | | **$** | **18,781,088** |

*Figure 19.5.* Fixed Cost Summary for the Company

## 19.6 Working Capital

*Figure 19.6* shows the total working capital investment for the company. The calculation parameters are the same as those in the CMO spreadsheet. Our total capital investment for working capital comes out to $553 million.

**Working Capital**

| | | 2020 | 2021 | 2022 |
|---|---|---|---|---|
| | Accounts Receivable | $ 295,890,411 | $ 147,945,205 | $ - |
| | Cash Reserves | $ 56,641 | $ 28,321 | $ - |
| | Accounts Payable | $ (34,333) | $ (17,167) | $ - |
| | Vaccine Inventory | $ 39,452,055 | $ 19,726,027 | $ - |
| | Raw Materials | $ 2,288 | $ 1,144 | $ - |
| | **Total** | **$ 335,367,062** | **$ 167,683,531** | **$ -** |
| | *Present Value at 15%* | *$ 385,672,121* | *$ 167,683,531* | *$ -* |
| **Total Capital Investment** | | | **$ 553,355,652** | |

*Figure 19.6.* Working Capital Summary for the Company

# 20. Other Important Considerations

## 20.1 Environmental Factors

*Gaseous Waste*

In bioreactors, cells require oxygen to grow and produce protein, releasing carbon dioxide in the process. Carbon dioxide, produced in excessive amounts, presents concerns with respect to limiting cell growth, protein production, and greenhouse gas emissions. To limit this concentration, the 500L and 2000L bioreactors will be equipped with spargers that feed nitrogen in the form of air to strip the carbon dioxide. Since we anticipate that the cells will produce carbon dioxide at a greater rate than it can be sparged, a base (sodium hydroxide, NaOH) will be added as needed to neutralize the excess carbonic acid that forms from the carbon dioxide in water. Dr. Cohen advised us that standard industrial practice is to vent bioreactor gases back into the room after the stream passes through a filter in the bioreactor. This gas stream contains oxygen, nitrogen, and carbon dioxide present in negligible amounts.

*Liquid Waste*

The upstream and downstream processes produce liquid waste streams that contain insect cells, cell debris, and inactivated viral particles. The upstream seed train produces four parallel streams of insect cells. One of these will be an extra stream in case of equipment malfunction or mechanical failure in one of the other lines, and will be treated with chlorine bleach to kill the live cells. The downstream purification processes produce large volumes of buffer and resins. The two chromatography columns use both resins and buffers that will contain larger cell debris and some viral particles. The diafiltration unit will produce buffers that contain small cellular components. These streams will also be treated with chlorine bleach. Dr. Cohen confirmed that

liquid streams treated with chlorine bleach can be disposed of as municipal waste poured down the drain.

*Solid Waste*

The upstream and downstream processes produce solid waste in the form of membrane filters that contain cell debris and inactivated viral particles. Two of the insect cell seed streams will be used in the baculovirus seed train, and the dead cell debris will be filtered out of the supernatant using a sterile filter. The depth filtration, virus filtration, and diafiltration units use membrane filters to separate out large and small cell debris and viral particles. These filters are treated as hazardous waste that will be disposed of in red biohazard bags. Consultation with Dr. Cohen revealed that solid biowaste is sent to a local incinerator that will burn the solids to ashes.

Many unit operations in the biopharmaceutical industry have single-use technologies to increase operation flexibility, reduce contamination risks, and eliminate the need for cleaning validation. This saves time and money by lessening the need for certain chemical resources and personnel. However, the tradeoff is the increased generation of plastic waste in the form of single-use plastic bioreactor bags and plastic piping. To minimize our plastic waste generation, we have designed the manufacturing process to produce and purify a sufficient amount of vaccines in one campaign.

## 20.2 Public and Employee Safety

*Baculovirus*

Our manufacturing process requires the use of a baculovirus to infect cells to produce the desired Spike protein. Despite being a virus, the baculovirus only infects insects and poses no harm to humans. It only contains the genetic information to make the Spike protein. There is no live handling of SARS-CoV-2, thus employees are in no danger of getting COVID-19 from this process. The process will also pose no biological threat to the public.

*Chemical Handling*

Different processes will require handling of acids, bases, and chemical powders. The viral inactivation operation requires the direct addition of acid and base, which will be connected to the mixer by piping. Large volumes of buffers for chromatography and diafiltration must be made by laboratory staff using various acids, bases, and powders. Any employee that handles these chemicals must be properly trained on the correct preparation and cleaning procedures, as well as be informed on any necessary emergency procedures.

## 20.3 Regulatory Requirements

The safety and efficacy of any vaccine must be scientifically tested and proven before being administered to the public. The data must be backed by the appropriate pre-clinical and clinical trials and undergo the necessary vetting and authorization processes for approval. The CMO facility must follow current good manufacturing practices (cGMPs) and meet the statutory and regulatory requirements for quality, development, manufacturing, and control. This will

ensure that the facility and equipment are sterile and qualified, and the potency and stability of the vaccine is maintained [14].

## 20.4 Social and Ethical Considerations

Company and CMO practices should ensure safe working conditions and environments that protect and support mental and physical health of employees, surrounding communities, and the public. In particular, employees must be properly trained to handle and dispose of waste. Additionally, allocation of vaccines should be done in a fair and equitable manner after manufacturing and formulation is complete. All nations should have access to the vaccine at an affordable price, regardless of wealth or global standing. COVAX (COVID-19 Vaccines Global Access) is a global initiative working toward this goal in collaboration with CEPI (Coalition for Epidemic Preparedness Innovation), GAVI (Gavi, the Vaccine Alliance, previously the Global Alliance for Vaccines and Immunization), WHO (World Health Organization), and other organizations with community outreach programs. Local distribution should prioritize recipients of vaccines based on their respective priority groups. Public health departments should work to mitigate health inequities for vulnerable populations to remove unjust, unfair, or unavoidable barriers to health and well-being [98].

## 20.5 Formulation and Packaging

The drug substance in the final storage tank after the last stage of the downstream process comprises 2.6 kg of recombinant spike protein with a buffer volume of 401.7 L. According to the

clinical study protocol for Novavax's SARS-CoV-2 recombinant protein vaccine, the dose level is 5 μg of protein with 50 μg of Matrix-M1 saponin-based adjuvant (ratio of 1:10) in a 0.5 mL solution [99]. Since the Novavax vaccine utilizes similar technology to our project, their protein concentration was sufficient to use as an estimate. Therefore, 2.6 kg of spike protein would need to be formulated with 26 kg of adjuvant, and the total amount of additional volume required would be about 259,522 L, which includes components such as buffers, preservatives, stabilizers, and antibiotics. Before sending the drug substance for formulation of drug product and packaging in vials, the drug substance is typically frozen or lyophilized for stability.

## 20.6 Batch Sizes

The number and size of production bioreactors are important design choices. One large bioreactor may have lower capital costs, but multiple smaller reactors likely have less financial losses due to quality concerns in the production bioreactor. According to a recent article on SARS-CoV-2 vaccine manufacturing, 15 million vaccine doses were discarded because workers used the incorrect ingredients [100]. If multiple smaller batches were used for the vaccine manufacturing process, less vaccine doses would have been wasted. However, because SARS-CoV-2 is an emergency measure and time is critical, there would be a bigger life threat if vaccines are developed more slowly. Thus, we believe that if workers are vigilant, with ready oversight provided on the factory floor, it is reasonable to choose a single, large batch for our production of COVID-19 vaccine to achieve timely production. Also, the 2000 L production bioreactor in our process design is relatively small compared to larger bioreactors currently in operation at various CMOs. Of course, if needed, our process can be divided into multiple small

batches run over a longer period of time to alleviate any financial concerns surrounding the investment of capital into a single large batch.

# 21. Profitability Analysis

## 21.1 CMO Profitability

Two profitability analyses were concurrently used to evaluate this project in consultation with Professor Bruce Vrana. The first analysis considered fixed costs for the CMO while the second analysis assessed the variable costs specific to this manufacturing process for our company. The CMO facility was assumed to have been designed in 2015 and constructed in 2016, with production and sales starting in 2017. The plant was assumed to operate at 40% capacity in 2017, 60% capacity in 2018, and 80% capacity from 2019 onwards. The COVID-19 vaccine production process is set to operate in 2021, where the CMO would be fully operational at 80% capacity.

To account for additional business that the CMO is involved in, the plant was assumed to manufacture 12 products per year for 15 years, with each product priced at a total of $1.15 million. The fixed costs under the CMO included equipment, labor, maintenance, and technical assistance. The CMO was assumed to charge a multiple of 3 times more than the total bare module cost for our equipment, in addition to a profit and in consideration of CMO competition for another factor of 2. This multiplying factor was taken into account in the CMO rental fee cost for our company to produce one campaign of vaccines. The CMO cash flow summary in *Figure 21.1* was generated under the mentioned assumptions using a 2% general inflation rate and a discount rate of 15% for capital.

Cash Flow Summary

| Year | Percentage of Design Capacity | Product Unit Price | Sales | Capital Costs | Working Capital | Var Costs | Fixed Costs | Depreciation | Depletion Allowance | Taxible Income | Taxes | Net Earnings | Cash Flow | Cumulative Net Present Value at 15% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2015 | 0% | | - | - | - | - | - | - | - | - | - | - | - | - |
| 2016 | 0% | | - | (6,759,700) | (743,400) | - | - | - | - | - | - | - | (7,503,100) | (6,524,400) |
| 2017 | 40% | $1,150,000.00 | 5,520,000 | - | (371,700) | (637,600) | (6,970,800) | (1,149,600) | - | (3,237,900) | 1,198,000 | (2,039,900) | (1,262,000) | (7,478,600) |
| 2018 | 60% | $1,173,000.00 | 8,445,600 | - | (371,700) | (975,500) | (7,110,200) | (1,839,400) | - | (1,479,400) | 547,400 | (932,000) | 535,600 | (7,126,400) |
| 2019 | 80% | $1,196,460.00 | 11,486,000 | - | - | (1,326,600) | (7,252,400) | (1,103,600) | - | 1,803,400 | (667,200) | 1,136,100 | 2,239,700 | (5,845,900) |
| 2020 | 80% | $1,220,389.20 | 11,715,700 | - | - | (1,353,200) | (7,397,400) | (662,200) | - | 2,303,000 | (852,100) | 1,450,900 | 2,113,000 | (4,795,300) |
| 2021 | 80% | $1,244,796.98 | 11,950,100 | - | - | (1,380,200) | (7,545,400) | (662,200) | - | 2,362,300 | (874,000) | 1,488,200 | 2,150,400 | (3,865,600) |
| 2022 | 80% | $1,269,692.92 | 12,189,100 | - | - | (1,407,800) | (7,696,300) | (331,100) | - | 2,753,800 | (1,018,900) | 1,734,900 | 2,066,000 | (3,088,900) |
| 2023 | 80% | $1,295,086.78 | 12,432,800 | - | - | (1,436,000) | (7,850,200) | - | - | 3,146,600 | (1,164,200) | 1,982,400 | 1,982,400 | (2,440,900) |
| 2024 | 80% | $1,320,988.52 | 12,681,500 | - | - | (1,464,700) | (8,007,200) | - | - | 3,209,500 | (1,187,500) | 2,022,000 | 2,022,000 | (1,866,100) |
| 2025 | 80% | $1,347,408.29 | 12,935,100 | - | - | (1,494,000) | (8,167,400) | - | - | 3,273,700 | (1,211,300) | 2,062,500 | 2,062,500 | (1,356,300) |
| 2026 | 80% | $1,374,356.45 | 13,193,800 | - | - | (1,523,900) | (8,330,700) | - | - | 3,339,200 | (1,235,500) | 2,103,700 | 2,103,700 | (904,100) |
| 2027 | 80% | $1,401,843.58 | 13,457,700 | - | - | (1,554,400) | (8,497,300) | - | - | 3,406,000 | (1,260,200) | 2,145,800 | 2,145,800 | (503,100) |
| 2028 | 80% | $1,429,880.45 | 13,726,900 | - | - | (1,585,500) | (8,667,300) | - | - | 3,474,100 | (1,285,400) | 2,188,700 | 2,188,700 | (147,400) |
| 2029 | 80% | $1,458,478.06 | 14,001,400 | - | - | (1,617,200) | (8,840,600) | - | - | 3,543,600 | (1,311,100) | 2,232,500 | 2,232,500 | 168,200 |
| 2030 | 80% | $1,487,647.63 | 14,281,400 | - | - | (1,649,500) | (9,017,400) | - | - | 3,614,500 | (1,337,400) | 2,277,100 | 2,277,100 | 448,000 |
| 2031 | 80% | $1,517,400.58 | 14,567,000 | - | 1,486,700 | (1,682,500) | (9,197,800) | - | - | 3,686,800 | (1,364,100) | 2,322,700 | 3,809,400 | 855,100 |

*Figure 21.1.* Cash Flow Summary for the CMO

Operation of the CMO facility is profitable, with the capital investment recovered within the 15 year production timeline. *Figure 21.2* shows that the plant has a net present value (NPV) of $855,000, a return on investment (ROI) of 18.08%, and an investor's rate of return (IRR) of 16.83%. Since the IRR is above 15%, this plant is considered profitable and attractive. These numbers motivate the operation of this plant through 2031, which ensures the facility can be operational in 2021 for the manufacture of our vaccine.

Profitability Measures

| | | |
|---|---|---|
| **The Internal Rate of Return (IRR) for this project is** | | 16.83% |
| **The Net Present Value (NPV) of this project in 2015 is** | $ | 855,100 |

**ROI Analysis (Third Production Year)**

| | |
|---|---|
| Annual Sales | 11,486,016 |
| Annual Costs | (8,579,031) |
| Depreciation | (540,775) |
| Income Tax | (875,498) |
| Net Earnings | 1,490,713 |
| Total Capital Investment | 8,246,422 |
| ROI | 18.08% |

*Figure 21.2.* Profitability Measures for the CMO

*Figure 21.3* shows a sensitivity analysis on IRR for the CMO based on the price per project product and the variable costs. Clearly, decreasing variable costs and/or increasing product price would result in a higher IRR. Since the CMO's variable costs of a project are paid by the partnering company, its own variable costs can only be reduced by selling and transferring expenses, research, and administrative expenses. The product price of $1.15 million per project

is an arbitrary number, the CMO can absolutely bring in more revenue by increasing the fee that partnering companies are charged. However, dropping the product price below $1.15 million or increasing variable costs by about $500,000 to $2 million would cause the IRR to dip below 15%, rendering the plant unattractive for investment. If the CMO consistently operates with factors that result in an IRR of at least 15%, the CMO would be more open to negotiating with our company on manufacture of the vaccine.

| Product Price \ Variable Costs | $796,950 | $956,340 | $1,115,730 | $1,275,120 | $1,434,510 | ***$1,593,900*** | $1,753,290 | $1,912,680 | $2,072,070 | $2,231,460 | $2,390,850 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $575,000 | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR |
| $690,000 | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR |
| $805,000 | -9.78% | -12.65% | -16.17% | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR |
| $920,000 | 4.83% | 3.59% | 2.28% | 0.90% | -0.58% | -2.18% | -3.91% | -5.82% | -7.97% | -10.43% | -13.35% |
| $1,035,000 | 13.74% | 12.81% | 11.87% | 10.89% | 9.89% | 8.86% | 7.80% | 6.70% | 5.56% | 4.36% | 3.11% |
| ***$1,150,000*** | 20.92% | 20.13% | 19.32% | 18.50% | 17.67% | 16.83% | 15.97% | 15.10% | 14.21% | 13.30% | 12.37% |
| $1,265,000 | 27.27% | 26.54% | 25.81% | 25.07% | 24.33% | 23.57% | 22.81% | 22.05% | 21.27% | 20.49% | 19.69% |
| $1,380,000 | 33.10% | 32.42% | 31.74% | 31.05% | 30.35% | 29.66% | 28.95% | 28.25% | 27.53% | 26.81% | 26.09% |
| $1,495,000 | 38.60% | 37.95% | 37.30% | 36.64% | 35.98% | 35.32% | 34.65% | 33.99% | 33.31% | 32.64% | 31.96% |
| $1,610,000 | 43.86% | 43.23% | 42.59% | 41.96% | 41.33% | 40.69% | 40.05% | 39.41% | 38.76% | 38.12% | 37.47% |
| $1,725,000 | 48.91% | 48.30% | 47.69% | 47.07% | 46.45% | 45.84% | 45.22% | 44.60% | 43.97% | 43.35% | 42.72% |

*Figure 21.3.* Sensitivity Analysis of IRR Using Product Price and CMO Variable Costs.

*Figure 21.4* shows a sensitivity analysis on IRR for the CMO based on the price per project product and the general inflation rate. Lower inflation rates and/or increasing product price would result in a higher IRR. However, dropping the product price below $1.15 million or having the inflation climb above 2.60% would cause the IRR to dip below 15%, rendering the plant unattractive for investment and closed to partnerships with companies like ours.

| Product Price \ Inflation | 1.00% | 1.20% | 1.40% | 1.60% | 1.80% | ***2.00%*** | 2.20% | 2.40% | 2.60% | 2.80% | 3.00% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $575,000 | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR |
| $690,000 | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR |
| $805,000 | -8.47% | -10.76% | -13.70% | -17.76% | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR | Negative IRR |
| $920,000 | 3.95% | 2.99% | 1.92% | 0.74% | -0.61% | -2.18% | -4.04% | -6.38% | -9.51% | -14.40% | Negative IRR |
| $1,035,000 | 12.36% | 11.75% | 11.10% | 10.40% | 9.66% | 8.86% | 8.00% | 7.05% | 6.00% | 4.83% | 3.48% |
| ***$1,150,000*** | 19.31% | 18.86% | 18.38% | 17.89% | 17.37% | 16.83% | 16.26% | 15.66% | 15.02% | 14.33% | 13.60% |
| $1,265,000 | 25.51% | 25.15% | 24.77% | 24.39% | 23.99% | 23.57% | 23.14% | 22.70% | 22.23% | 21.75% | 21.24% |
| $1,380,000 | 31.25% | 30.95% | 30.64% | 30.32% | 29.99% | 29.66% | 29.31% | 28.95% | 28.58% | 28.20% | 27.81% |
| $1,495,000 | 36.68% | 36.42% | 36.16% | 35.88% | 35.60% | 35.32% | 35.03% | 34.73% | 34.42% | 34.11% | 33.79% |
| $1,610,000 | 41.88% | 41.65% | 41.42% | 41.18% | 40.94% | 40.69% | 40.44% | 40.18% | 39.92% | 39.65% | 39.37% |
| $1,725,000 | 46.90% | 46.69% | 46.49% | 46.27% | 46.06% | 45.84% | 45.61% | 45.39% | 45.16% | 44.92% | 44.68% |

*Figure 21.4.* Sensitivity Analysis of IRR Using Product Price and General Inflation Rate.

## 21.2 Company Profitability

The company cash flow summary in *Figure 21.5* was generated using a selling price of $16 per dose [94]. Inflation was considered negligible in the short timespan of one campaign,

and a discount rate of 15% for capital was used. Since the scope of the project is limited to the manufacture of 500 million vaccine doses, company cash flows were not considered outside of this campaign. Therefore, the cash flow summary only has valid inputs for the first year. From one production campaign, the project is estimated to have a net present value of $1.5 billion dollars, with positive net earnings of $2 billion and cash flow of $1.5 billion in the same year as production. However, the short lifespan of this project was difficult to incorporate into the profitability analysis, which is typically done for much longer projects, such as in the case of the proposed CMO facility.

Cash Flow Summary

| Year | Percentage of Design Capacity | Product Unit Price | Sales | Capital Costs | Working Capital | Var Costs | Fixed Costs | Depreciation | Depletion Allowance | Taxible Income | Taxes | Net Earnings | Cash Flow | Cumulative Net Present Value at 15% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2021 | 45% | $16.00 | 3,600,000,000 | - | (503,050,600) | (416,217,700) | (18,781,100) | - | - | 3,165,001,200 | (1,171,050,400) | 1,993,950,700 | 1,490,900,200 | 1,490,900,200 |
| 2022 | 68% | #N/A | #N/A | - | 503,050,600 | (624,326,600) | (18,781,100) | - | #N/A | #N/A | #N/A | #N/A | #N/A | #N/A |

*Figure 21.5.* Cash Flow Summary for the Company

None of the previous IRR, NPV, or ROI values could be calculated, as our IRR would appear ridiculously large. Dr. Arthur Etchells observed that this was due to the fact that our company had no investment input for bare module costs, direct permanent investment, depreciable capital, or total permanent investment. Despite the presence of variable costs, fixed costs, and working capital investments, the spreadsheet was unable to determine any of the profitability values for the company. Dr. Koshari reminded the team that typically there are years of research and development (R&D), pre-clinical, and clinical trial costs invested in prior years, which were not accounted for in this spreadsheet. There is also a portion of profit that formulation and packaging takes after completion of manufacture, which would decrease the profits of the company but were not accounted for. Due to these complications, a sensitivity analysis on the vaccine selling price could not be performed. However, a project worth $1.5 billion for the one year it is implemented is clearly very profitable.

# 22. Conclusions and Recommendations

The manufacture of 500 million doses of recombinant SARS-CoV-2 spike protein-based vaccines using BEVS is economically profitable for both the CMO and our company. We will achieve an extremely large IRR, as our permanent investment is negligible, as this cost will be taken up by the CMO. The IRR for the CMO plant is 16.83% assuming the CMO charges us $1.15 million per month to use their facilities. Our company will then incur the variable costs, working capital costs, and the rental fee for the CMO. Charging $16 per dosage of vaccine results in $8 billion in revenue. Our net earnings for the process accumulate to be about $2 billion. Hence, the project is extremely profitable and we recommend the manufacture of this vaccine immediately for emergency use.

Overall, the process is expected to produce 3.75 kg of spike protein in the upstream process with a 30% yield loss in the downstream process, producing a final drug substance of 2.6 kg of spike protein. The upstream process takes 24 days to complete, the downstream process about 3 days. Since the overall process is about 27 days, a month should be an effective estimate of production time. While multiple production cycles are not anticipated, a total of four cycles could be anticipated to reach completion within the course of 3 months, assuming that each cycle starts midway through the prior cycle. The process has low energy requirements due to low flow rates needed to safely operate and maintain both upstream and downstream processes at room temperature and pressure. The main drivers of energy costs are the operations of the perfusion and production bioreactors.

As a next step, we recommend completing an economic analysis under the assumption that COVID-19 vaccines will be needed on an annual basis like the flu vaccine. Currently, scientists are unsure if yearly vaccination will be required, if a booster will be necessary, or if

immunity from a single vaccine will be sufficient. Optimally a vaccine would not be needed yearly, but we recognize the likelihood of this possibility. Thus, the consideration of long-term operation would be fruitful, either building a company facility to produce vaccines in-house, or establishing a years-long contract with a CMO. Secondly, this project is designed for one campaign, but we recommend supplemental analyses for smaller manufacturing batches over a longer production period. Finally, we recommend that our company includes the R&D investments and considers profits lost to formulation and processing, which are outside the scope of this project but would provide a more accurate assessment of the economics of this project.

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# 25. Appendix

## A. Supplemental Calculations

### *A.1 Cellular Growth Kinetics*

Cell growth in exponential phase is defined as:

$$\frac{dx}{dt} = \mu x \quad \{6\}$$

$$\frac{dx}{x} = \mu \cdot dt \quad \{7\}$$

$$\int \frac{dx}{x} = \mu \cdot \int dt \quad \{8\}$$

$$ln \frac{x}{x_o} = \mu \cdot \Delta t \quad \{9\}$$

where $\mu$ = specific cell growth rate
$x_o$ = initial cell population
$x$ = final cell population
$\Delta t$ = batch time

As previously mentioned in Section 11.3, the specific cell growth rate (μ) of Sf-9 cells is 0.033 $hr^{-1}$, which corresponds to a doubling time of 21 hr by using *Equation {9}*. Setting the batch times of each stage in the upstream process and knowing the initial cell population in the inoculum vial from the cell bank, the final number of cells in each stage can be calculated, again by using *Equation {9}*. Since the maximum VCD of Sf-9 cells is $8.1 \times 10^6$ cells/mL, the batch times were set accordingly so that the final cell population in each stage does not exceed the maximum VCD, except for the 500 L perfusion bioreactor as explained in Section 13.1 and Section 15.15. *Table 25.1* below summarizes the number of cells and batch times in each stage of the upstream process.

*Table 25.1.* Number of cells and batch times in each stage of the upstream process.

| | Stock Vial | 125 mL Flask | 500 mL Flask | 5 L Flask | 50 L WAVE | 500 L Brx | 2000 L Brx |
|---|---|---|---|---|---|---|---|
| Final VCD ($\times 10^6$ cells/mL) | 4.05 | 4.98 | 5.36 | 5.78 | 6.22 | 32.65 | 8.15 |
| Working Volume (L) | 0.001 | 0.05 | 0.5 | 5 | 50 | 500 | 2000 |
| Initial # of Sf-9 Cells | - | 4.05E6 | 2.49E8 | 2.68E9 | 2.89E10 | 3.11E11 | 1.63E13 |
| Final # of Sf-9 Cells | 4.05E6 | 2.49E8 | 2.68E9 | 2.89E10 | 3.11E11 | 1.63E13 | - |
| Batch Time (Days) | - | 5.2 | 3 | 3 | 3 | 5 | 5 |

### *A.2 Gas-Liquid Mass Transfer Coefficient*

Gas-liquid mass transfer coefficient ($k_La$) is a measurement of the efficiency with which the bioreactor is capable of delivering oxygen into or stripping carbon dioxide from cell culture. The most critical variable that affects $k_La$ is aeration rate of spargers; agitation rate of impellers does not affect $k_La$ much. In order to make sure whether a bioreactor can support corresponding aeration rates, $k_La$ values for oxygen delivery and carbon dioxide stripping must be calculated.

The oxygen uptake rate (OUR) is defined as:

$$OUR = sOUR \cdot VCD \qquad \{10\}$$

where $OUR$ = oxygen uptake rate
$sOUR$ = specific oxygen uptake rate
$VCD$ = viable cell density

As previously mentioned in Section 11.4, the specific oxygen uptake rate is 0.22 to 0.26 μM $O_2/10^6$ cell/hr during the growth phase [63] and 0.2 to 0.76 μM/$10^6$ cell/hr for the infection phase [37]. For the 500 L perfusion bioreactor that has cells in growth phase, 0.26 μM $O_2/10^6$ cell/hr was used as the sOUR value. For the 2000 L production bioreactor that has cells in

infection phase, 0.76 µM $O_2/10^6$ cell/hr was used as the sOUR value. The upper bounds for the sOUR values were used as a conservative approach. Final VCD values of the perfusion and production bioreactors in Table 25.1 were used for *Equation {10}* calculations.

The oxygen transfer rate (OTR) is defined as:

$$OTR = k_L a_{O2} \cdot (p_{O2,liq} - p_{O2,gas}) \cdot H_{O2/H2O} \qquad \{11\}$$

where
$OTR$ = $O_2$ Transfer Rate
$k_L a_{O2}$ = $O_2$ Gas-Liquid Mass Transfer Coefficient
$p_{O2}$ = $O_2$ Partial Pressure in Liquid or Gas
$H_{O2/H2O}$ = Henry's Law Constant of $O_2$ in Water

As previously mentioned in Section 11.4, cells were grown in 65% $O_2$ concentration and infected in 50% $O_2$ concentration, which correspond to the $p_{O2,liquid}$ values of the 500 L perfusion bioreactor and 2000 L production bioreactor, respectively. For the $p_{O2,gas}$ value of the perfusion bioreactor, gas sparged through the frit sparger for oxygen delivery was set to have 50% $O_2$. For the $p_{O2,gas}$ value of the production bioreactor, gas sparged through the frit sparger for oxygen delivery was set to have 100% $O_2$. Henry's law constant of $O_2$ in water ($H_{O2/H2O}$) can be found from *Figure 25.1* below. The $H_{O2/H2O}$ values for both the perfusion bioreactor at 27.5˚C and the production bioreactor at 26˚C are about 0.037.

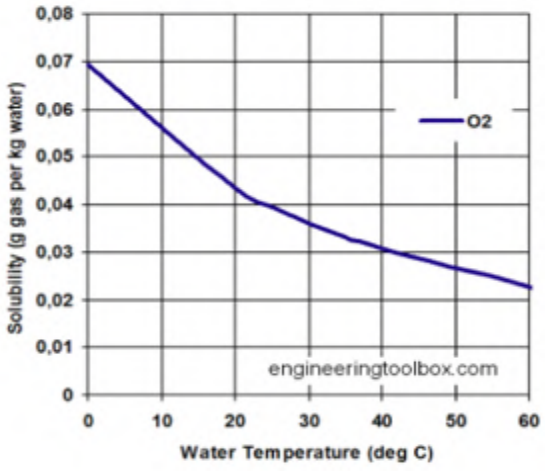
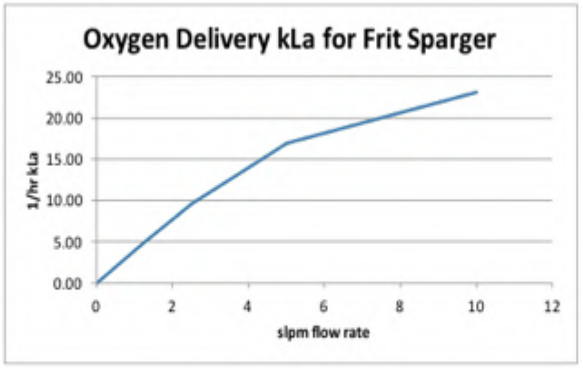
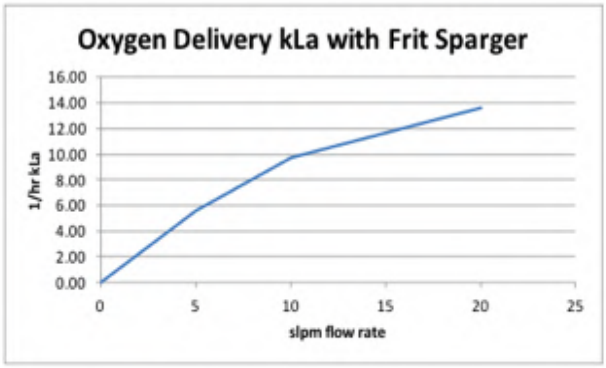



*Figure 25.1.* Henry's law constant of $O_2$ in Water as a function of temperature. (Engineering Toolbox)

By setting the OUR in *Equation {10}* and OTR in *Equation {11}* equal to each other, oxygen delivery $k_La$ ($k_La_{O2}$) can be calculated. The required $k_La_{O2}$ for the 500 L perfusion bioreactor is 20.16 $hr^{-1}$, and that for the 2000 L production bioreactor is 5.98 $hr^{-1}$. From *Figure 25.2* below, we can see that the corresponding aeration rate of oxygen delivery for the perfusion bioreactor is 8 SLPM, and that for the production bioreactor is 6 SLPM.




**Graph 2.17** Interpolated Results for 500L S.U.B. with porous frit sparger




**Graph 2.57** Results for 2,000L S.U.B. with porous frit sparger

*Figure 25.2.* Oxygen delivery $k_La$ with frit sparger for the perfusion and production bioreactors. (Thermo Scientific HyPerforma Single-Use Bioreactor Systems Validation Guide)

The carbon dioxide evolution rate (CER) is defined as:

$$CER = sOUR \cdot VCD \cdot RQ \qquad \{12\}$$

where
$CER$ = carbon dioxide evolution rate
$sOUR$ = specific oxygen uptake rate
$VCD$ = viable cell density
$RQ$ = respiratory quotient (mol $CO_2$/mol $O_2$)

The sOUR and VCD values used for *Equation {12}* calculations are the same as those used for *Equation {10}* calculations. As described in Section 11.4, the respiratory quotient (RQ) for uninfected Sf-9 cells is 1.07 ± 0.06, and that for virus-infected cells is 1.12 ± 0.03 [76]. Therefore, the RQ value used for the 500 L perfusion bioreactor was 1.07, and that used for the 2000 L production bioreactor was 1.12.

The carbon dioxide transfer rate (CTR) is defined as:

$$CTR = k_L a_{CO2} \cdot (p_{CO2,liq} - p_{CO2,gas}) \cdot H_{CO2/H2O} \quad \{13\}$$

where
$CTR$ = $CO_2$ transfer rate
$k_L a_{CO2}$ = $CO_2$ gas-liquid mass transfer coefficient
$p_{CO2}$ = $CO_2$ partial pressure in liquid or gas
$H_{CO2/H2O}$ = Henry's law constant of $CO_2$ in water

As described in Section 11.2, the $p_{CO2,liq}$ level is set at 30 mmHg for both the perfusion and production bioreactors. For the $p_{CO2,gas}$ values of both bioreactors, the air sparged through the drilled hole sparger for carbon dioxide stripping was assumed to have 0 mmHg $CO_2$. Henry's law constant of $CO_2$ in water ($H_{CO2/H2O}$) can be found from *Figure 25.3* below. The $H_{CO2/H2O}$ values for the perfusion bioreactor at 27.5˚C and the production bioreactor at 26˚C are both around 1.35.

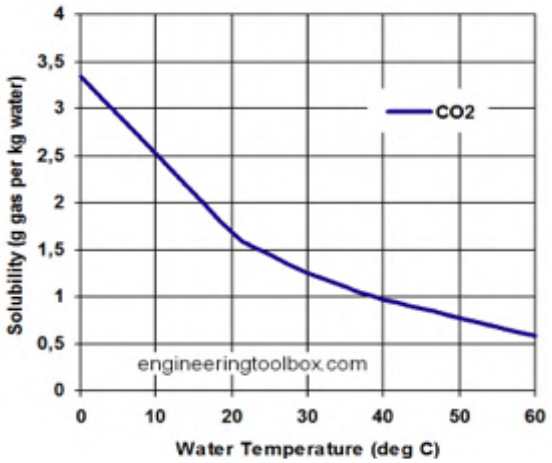
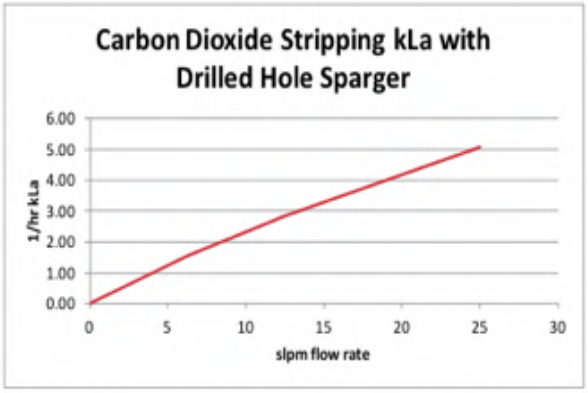


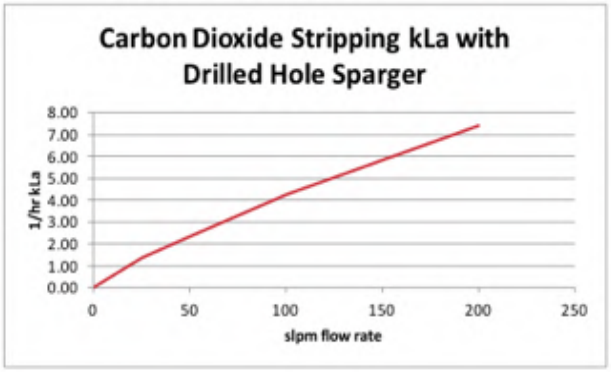

*Figure 25.3.* Henry's law constant of $CO_2$ in Water as a function of temperature. (Engineering Toolbox)

By setting the CER in *Equation {11}* and CTR in *Equation {13}* equal to each other, $CO_2$ stripping $k_La$ ($k_La_{CO2}$) can be calculated. The required $k_La_{CO2}$ for the 500 L perfusion bioreactor is 7.5 $hr^{-1}$, and that for the 2000 L production bioreactor is 5.73 $hr^{-1}$. $CO_2$ stripping is a composite mass transfer process, where both the air supply from drilled hole sparger and the oxygen supply from frit sparger strip carbon dioxide. From *Figure 25.4* and *Figure 25.5* below, we can see that

the 500 L perfusion bioreactor requires an aeration rate of 25 SLPM through the drilled hole sparger ($k_La_{CO2}$ of 5 $hr^{-1}$) with the aeration rate of 8 SLPM through the porous frit sparger ($k_La_{CO2}$ of 2.5 $hr^{-1}$) mentioned earlier in the $K_La_{O2}$ analysis. Also, we can see that the 2000 L production bioreactor needs an aeration rate of about 150 SLPM through the drilled hole sparger ($k_La_{CO2}$ of 5.7 $hr^{-1}$) with the aeration rate of 6 SLPM through the frit sparger ($k_La_{CO2}$ of 0.5 $hr^{-1}$), again mentioned earlier in the $K_La_{O2}$ analysis.

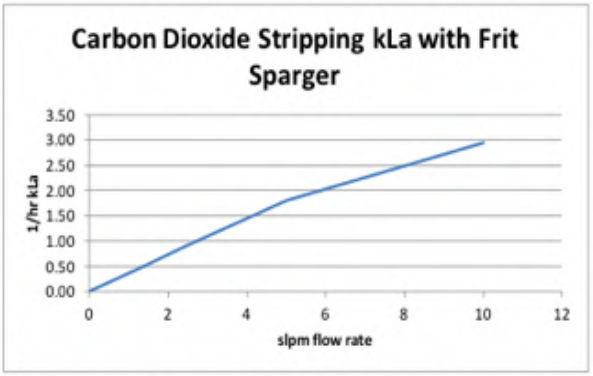



**Graph 2.50** Interpolated Results for 500L S.U.B. with drilled hole sparger

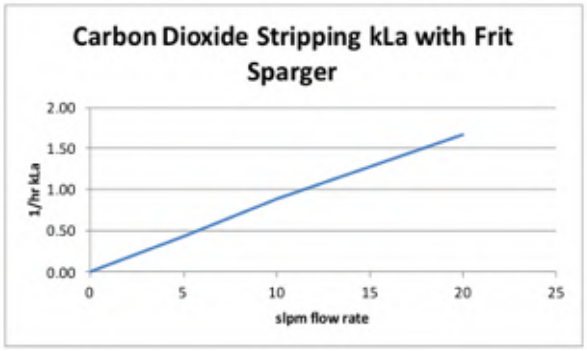



**Graph 2.60** Results for 2,000L S.U.B. with drilled hole sparger

*Figure 25.4.* $CO_2$ stripping $k_La$ with DH sparger for the perfusion and production bioreactors. (Thermo Scientific HyPerforma Single-Use Bioreactor Systems Validation Guide)




**Graph 2.49** Interpolated Results for 500L S.U.B. with porous frit sparger




**Graph 2.59** Results for 2,000L S.U.B. with porous frit sparger

*Figure 25.5.* $CO_2$ stripping $k_La$ with frit sparger for the perfusion and production bioreactors. (Thermo Scientific HyPerforma Single-Use Bioreactor Systems Validation Guide)

### *A.3 Kolmogorov Eddy Length*

Engineers hypothesize that if Kolmogorov eddy length is smaller than the cell diameter, the cells may be sheared and die. In other words, as long as the Kolmogorov eddy length is larger than the cell diameter, the cells would not be in danger of shearing. If the calculated Kolmogorov eddy length turns out to be smaller than the cell diameter, the agitation rate of the impeller needs to be slowed down to increase the eddy size.

The Kolmogorov eddy length is defined as:

$$\eta = \left(\frac{\nu^3}{\varepsilon}\right)^{1/4} \quad \{14\}$$

where $\eta$ = Kolmogorov length scale
$\nu$ = kinematic viscosity
$\varepsilon$ = agitation power / mass

Kinematic viscosity ($\nu$) of media was assumed to be properties of water, since the media solution is mostly aqueous and very dilute. The mass in ε was assumed to be the spherical mass of the media that directly contacts the impeller.

To calculate the agitation power (P) in ε:

$$N_P = \frac{P}{\varrho \cdot n^3 \cdot D^5} \quad \{15\}$$

where $N_P$ = power number
$P$ = agitation power
$\rho$ = fluid density
$n$ = agitation rate
$D$ = impeller diameter

According to the specifications sheets provided by Thermo Fisher Scientific, the power number ($N_P$), impeller diameter (D), and nominal agitation rate (n) are each given for both 500 L

perfusion and 2000 L production bioreactors. Fluid density (ρ) of media was assumed to be the same as water density, again because the solution is mostly aqueous and very dilute.

*Table 25.3* below summarizes Kolmogorov eddy lengths of both the perfusion and production bioreactors, calculated by using *Equation {14}* and *Equation {15}* shown above. Since the calculated Kolmogorov eddy lengths for both bioreactors are larger than the average diameter of Sf-9 cells, which is 18 μm [101], the nominal agitation rates are safe for usage.

*Table 25.3.* Kolmogorov eddy lengths of the perfusion and production bioreactors

| | 500 L Perfusion Bioreactor | 2000 L Production Bioreactor |
|---|---|---|
| Agitation Rate (n) | 101 RPM | 75 RPM |
| Power Number ($N_P$) | 2.1 | 2.1 |
| Impeller Diameter (D) | 25.1 cm | 39.8 cm |
| Fluid Density (ρ) | 1 g/mL | 1 g/mL |
| Kinematic Viscosity ($\nu$) | 1 cp | 1 cp |
| **Kolmogorov Eddy Length (η)** | **28 μm** | **28 μm** |

### *A.4 Mixing Time*

After determining whether the nominal agitation rate is safe for usage by calculating the Kolmogorov eddy lengths, mixing time to reach 95% homogeneity ($\theta_{95}$) must also be considered, since cells need to be in bioreactors with good mixing for even supply of nutrients. Typically, $\theta_{95}$ of less than 1 minute is considered good mixing, suggested by our project author Dr. Cohen.

To calculate $\theta_{95}$, Equation (9-1) in Handbook of Industrial Mixing (2004) was used [102]:

$$N_P^{1/3} \cdot n \cdot \theta_{95} \cdot \frac{D^2}{T^{1.5} \cdot H^{0.5}} = 5.20 \qquad \{16\}$$

where
$N_P$ = power number
$n$ = agitation rate
$\theta_{95}$ = mixing time to reach 95% homogeneity
$D$ = impeller diameter
$T$ = tank inner diameter
$H$ = fluid height

Again, the power number ($N_P$), impeller diameter (D), as well as tank inner diameter (T) are provided by the Thermo Fisher Scientific specifications sheets for both 500 L perfusion and 2000 L production bioreactors. The same agitation rate (n) values used to check the Kolmogorov eddy lengths previously were used. Fluid height (H) was calculated by using the working volume and tank inner diameter for each bioreactor.

*Table 25.4* below summarizes the mixing time to reach 95% homogeneity for both the perfusion and production bioreactors, calculated by using *Equation {16}* above. Since $\theta_{95}$ for both bioreactors are less than 1 minute, the agitation rates are sufficient to provide good mixing.

*Table 25.4.* Mixing time to reach 95% homogeneity for the perfusion and production bioreactors

| | 500 L Perfusion Bioreactor (Working Volume: 500 L) | 2000 L Production Bioreactor (Working Volume: 2000 L) |
|---|---|---|
| Agitation Rate (n) | 101 RPM | 75 RPM |
| Power Number ($N_P$) | 2.1 | 2.1 |
| Impeller Diameter (D) | 25.1 cm | 39.8 cm |
| Tank Inner Diameter (T) | 75.6 cm | 119.4 cm |
| Fluid Height (H) | 111.4 cm | 178.6 cm |
| **Mixing Time to Reach 95% Homogeneity ($\theta_{95}$)** | **27 seconds** | **36 seconds** |

## B. Major Literature Sources

## C. Vendor Specification Sheets

gibco
by life technologies

# Sf-900™ II SFM

## Description

Sf-900™ II SFM is a complete, serum-free, protein-free, ready-to-use insect cell culture medium developed for high cell-density growth and high-level recombinant protein expression using the Baculovirus Expression Vector System (BEVS). Sf-900™ II SFM has optimized amino acid, carbohydrate, vitamin, and lipid components, as well as a biologically active raw material providing significant improvement in cell growth, virus production and recombinant protein expression over other serum-free or serum-supplemented media. Sf-900™ II SFM supports long term growth (>20 passages) of *Spodoptera frugiperda* (Sf9, Sf21), *Trichoplusia ni* (Tn-368), and *Lymantia dispar* (Ld) cells in both suspension and monolayer culture.

| Product | Catalog No. | Amount | Storage | Shelf life* |
|---|---|---|---|---|
| Sf-900™ II SFM (1X), liquid | 10902-096<br>10902-153<br>10902-088<br>10902-104 | 500 mL<br>10 × 500 mL<br>1000 mL<br>6 × 1000 mL | 2°C to 8°C; Protect from light | 12 months |
| Sf-900™ II SFM (1X), liquid, Universal Bag | 10902-161<br>10902-179<br>10902-187 | 5 L<br>10 L<br>20 L | 2°C to 8°C; Protect from light | 12 months |
| Sf-900™ II SFM (1X), liquid w/out methionine or cysteine | 21012-026 | 500 mL | 2°C to 8°C; Protect from light | 12 months |

* Shelf life duration is determined from Date of Manufacture.

## Product use

For Research Use Only. Not for use in diagnostic procedures.

## Important information

Sf-900™ II SFM is a complete, ready to use medium. Do not add L-Glutamine or surfactants such as Pluronic® F-68.

## Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Culture Conditions

**Media:** Sf-900™ II SFM

**Cell line:** Sf9, Sf21, Ld, Tn-368 cells

**Culture type:** Suspension or Adherent

**Culture vessels:** Shake flask, spinner bottle or T-flask.

**Temperature range:** 27°C to 28°C

**Incubator atmosphere:** Non-humidified, air regulated non-$CO_2$ atmosphere. Ensure proper gas exchange and minimize exposure of cultures to light.

## Recovery

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Transfer entire contents of the cryovial into the appropriately sized vessel so that the cells are seeded at 3–5 × $10^5$ cells/mL of pre-warmed Sf-900™ II SFM.
3. Incubate at 28°C in a non-humidified, air regulated non-$CO_2$ atmosphere, on an orbital shaker platform rotating at 120–140 rpm. Loosen flask caps to allow for gas exchange.
4. Subculture when cells reach >2 × $10^6$ viable cells/mL. We recommend subculturing cells a minimum of 3 passages before using in downstream applications.

## Subculture suspension cultures

Insect cells are sensitive to physical shearing. Ensure that impeller mechanisms rotate freely and do not contact vessel walls or base (adjust prior to autoclaving).

1. Determine viable cell density using a Countess® Automated Cell Counter or alternative automated or manual method.
2. Seed cells at 3–5 × $10^5$ viable cells/mL in sterile culture vessels containing pre-warmed Sf-900™ II SFM. (30 mL per 125-mL shake flask, 75–100 mL per 100-mL spinner bottle).
3. Incubate at 27°C to 28°C in a non-humidified, air regulated non-$CO_2$ atmosphere. Loosen caps to allow for gas exchange.
4. Rotate shake flask cultures on an orbital shaker platform at 120–140 rpm, set impeller stirring rate to 85–95 rpm for spinner bottles (optimum impeller speed must be empirically determined for each spinner apparatus for robust cell growth and viability).
5. Subculture cells when viable cell density reaches >2 × $10^6$ viable cells/mL (about twice a week) into clean, sterile flask(s) with fresh pre-warmed Sf-900™ II SFM.

**Note:** If cell debris is observed, gently centrifuge the cell suspension at 100 × *g* for 5–10 minutes and resuspend the cell pellet in fresh Sf-900™ II SFM to reduce accumulation of cell debris and metabolic waste by-products.

**Note:** We recommend thawing a fresh low-passage vial of cells every 3 months or 30 passages.

## Subculture monolayer cultures

1. Observe cell monolayer to ensure 80–90% confluence. Aspirate medium and floating cells from a confluent monolayer.
2. Add 4 mL (per 25 $cm^2$) pre-warmed Sf-900™ II SFM to the flask and resuspend cells by repeatedly pipetting the medium across the monolayer.
3. Observe cell monolayer to ensure cell detachment from the surface of the flask. Firmly rap the side of the flask on the palm of your hand or a hard flat surface if necessary.

4. Transfer entire cell suspension to a sterile conical tube; any cell clumps quickly settle to the bottom after 1–2 minutes. Pipet the clumps into a 10-mL pipette and gently break up the clumps by pressing the pipette tip against the bottom of the tube and gently expell the cells back into the medium, repeat if necessary to break up remaining clumps. Pipetting too harshly will decrease cell viability due to sensitivity of cells to shear force.
5. Determine viable cell density using a Countess® Automated Cell Counter.
6. Inoculate 2–5 × $10^4$ viable cells/$cm^2$ into new culture flasks containing pre-warmed Sf-900™ II SFM (5 mL/25 $cm^2$).
7. Incubate at 27°C to 28°C in a non-humidified, air regulated non-$CO_2$ atmosphere. Loosen caps to allow for gas exchange.
8. Three days post-plating, aspirate medium from the cell monolayer and re-feed the culture with an equal volume of fresh medium gently added to the side of the flask.

**Note:** Sf9 cells are not anchorage dependent and may be transferred between monolayer and spinner/shaker culture repeatedly without noticeable change in viability, morphology, or growth rate.

## Adapt cells to Sf-900™ II SFM

It is critical that cell viability be ≥90% and the growth rate be in mid-logarithmic phase prior to initiating adaptation procedures.

### Direct adaptation

Monolayer cultures need only have the culture media exchanged with prewarmed Sf-900™ II SFM as described in **Subculture monolayer cultures.**

Transfer suspension cultures into Sf-900™ II SFM as follows:

1. Centrifuge the cell suspension at 100 × *g* for 5–10 minutes. Aspirate and discard the supernatant.
2. Resuspend the cell pellet in pre-warmed Sf-900™ II SFM at a viable cell density of >5 × $10^5$ cells/mL and transfer to appropriate culture vessel.
3. Return to incubator and monitor cell growth.

**Note:** If suboptimal performance is achieved using the direct adaptation method, use the sequential adaptation method.

### Sequential adaptation

Follow the procedures for subculture of suspension or monolayer cultures with the following modifications.

1. During the adaptation procedure use a seeding density of >5 × $10^5$ viable cells/mL.
2. Subculture cells into stepwise increasing ratios of Sf-900™ II SFM to original medium with each subsequent passage (25:75, 50:50, 75:25, 90:10 followed by 100% Sf-900™ II SFM). Multiple passages at each step may be needed.

After several passages in 100% Sf-900™ II SFM, the viable cell count should exceed 2–4 × $10^6$ cells/mL with a viability exceeding 85% within 4–6 days of culture.

## Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of >1 × $10^7$ cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% Sf-900™ II SFM (50:50 ratio of fresh to conditioned media) + 7.5% DMSO on day of intended use, store at 4°C until use.
4. Centrifuge cell suspension at 100 × *g* for 5–10 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this cell suspension into cryovials according to the manufacturer's specifications.
6. Cryopreserve in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen, (vapor phase) storage at –200°C to –125°C.

## Related products

| Product | Catalog no. |
|---|---|
| Sf9 Cells Adapted in Sf-900™ II SFM | 12659 |
| Sf21 Cells Adapted in Sf-900™ II SFM | 12682 |
| BaculoDirect™ N-Term Expression Kit | 12562-054 |
| BaculoDirect™ N-Term Transfection Kit | 12562-062 |
| BaculoDirect™ C-Term Expression Kit | 12562-013 |
| BaculoDirect™ C-Term Transfection Kit | 12562-039 |
| Bac-N-Blue™ Transfection Kit | K855-01 |
| Bac-to-Bac® Baculovirus Expression System | 10359 |
| Bac-to-Bac® Vector Kit | 10360 |
| Countess® Automated Cell Counter | C10227 |

## Explanation of symbols and warnings

The symbols present on the product label are explained below:

| | | | | |
|---|---|---|---|---|
|  |  |  |  |  |
| Temperature Limitation | Manufacturer | Batch code | Use By: | Catalog number |
|  |  |  |  | |
| Caution, consult accompanying documents | Consult instructions for use | Keep away from light | Sterilized using aseptic processing techniques | |

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**

# Cultivation of Sf9 insect cells and rSEAP expression in the HyPerforma Rocker Bioreactor

## Summary

This application note describes the cultivation of Sf9 suspension cells in the Thermo Scientific™ HyPerforma™ Rocker Bioreactor with a maximum working volume of 5.0 L. Using a serum-free medium without components of animal origin, viable cell densities of up to 6.2 x $10^6$ cells/mL were achieved. Expression of recombinant secreted alkaline phosphatase (rSEAP) was induced using a baculovirus expression vector system. rSEAP activity up to a maximum of 58.5 U/mL was achieved. The results are in the typical range observed in previous cultivations using wave-mixed bioreactor types.


**Figure 1. TruBio software with the HyPerforma G3Lab Controller and the HyPerforma Rocker Bioreactor.**

## Introduction

The HyPerforma Rocker Bioreactor (Figure 1) is a lab-scale rocker-type bioreactor that is characterized by low shear stresses and is often used for seed train production in biopharmaceutical production processes.

The HyPerforma Rocker Bioreactor can be controlled using the Thermo Scientific™ HyPerforma™ G3Lab™ Controller in conjunction with Thermo Scientific™ TruBio™ software powered by the Emerson™ DeltaV™ system (Figure 1).

The scope of the study was to demonstrate the applicability of the HyPerforma Rocker Bioreactor for cultivating a *Spodoptera frugiperda* (Sf9) insect cell line in a biphasic production process using serum-free medium and expressing a model protein, rSEAP. The production process was based on a baculovirus expression vector system (BEVS).

## Materials and methods

### Overview of procedure setup

- Day –6/–1: Inoculum production with Sf9 suspension cells in shaker flasks.
- Day 0: Bioreactor preparation, inoculation with seeding density of 1 x $10^6$ cells/mL in 3.0 L of Gibco™ Sf-900™ III SFM.
- Day 1: Sampling, starting of control loop for pH.
- Day 2: Sampling; addition of the virus suspension and fresh medium to achieve a final volume of 5.0 L and infected cell density (ICD) of 2 x $10^6$ cells/mL at a multiplicity of infection (MOI) of 0.01 PFU/cell.
- Day 3: Sampling, analytics, and harvesting. Production was stopped 7 days postinfection. This corresponds to a time of harvest (TOH) of 164 hours.

### Medium

Gibco™ Sf9 cells were cultivated in Sf-900 III SFM containing Gibco™ Pluronic™ F-68 and L-glutamine. The expression of rSEAP was induced by a baculovirus expression vector system (Sil9.1.1_GFP_SEAP_His MP 9.8 V2, provided by ZHAW IBT Molecular Biology) with a virus titer of 1.5 x $10^9$ PFU/cell.

### Inoculum preparation

The inoculum for the HyPerforma Rocker Bioreactor was produced in single-use 250 and 500 mL shaker flasks at working volumes of 100 and 200 mL. The cells (passage #33) were inoculated at cell densities of about 0.7–1.0 x $10^6$ cells/mL. The flasks were placed in a shaking incubator (Ecotron™, Infors™ HT, CH) at 27°C with a shaking rate of 100 rpm and an amplitude of 25 mm. A cell suspension of 1,200 mL with a cell density of approximately 3.3 x $10^6$ cells/mL was produced for inoculation of the HyPerforma Rocker Bioreactor.

### Bioreactor preparation

A NaOH solution was produced, put in Duran™ glass bottles, and autoclaved for 20 minutes at 121°C. The HyPerforma Rocker BioProcess Container (BPC) was filled with 1 L of the fresh medium using a sterile syringe connected to the luer lock connector, and placed on the rocking platform. After the aeration (0.25 slpm) and heating (27°C) were switched on, one-point calibration was performed for the pH sensor. A sample was taken and the pH was determined using an external pH meter (Mettler-Toledo). The $pO_2$ sensor was calibrated to 100% saturation.

### Inoculation preparation

The inoculation procedure was performed under a laminar flow, where the Duran glass bottle with the NaOH solution (0.1 M) was connected to the HyPerforma Rocker BPC via a luer lock connector directly before inoculation. In order to achieve the desired cell density of 1 x $10^6$ cells/mL, 0.95 L of the cell suspension was added to the bioreactor through a sterile funnel, followed by 1.05 L of fresh medium, to achieve the initial working volume of 3.0 L. Afterwards, the bioreactor was reconnected to the control unit and all the control loops for dissolved oxygen (DO) and temperature were started. The settings were defined as given below. The control loop for pH was started after 24 hours of process time.

### Process parameters

Temperature was controlled automatically via an integrated heater in the rocking platform. DO concentration was controlled using a cascade function with rocking rate as the primary factor, and the addition of pure oxygen as the secondary factor. To automatically control the pH during the growth phase, a control loop with the addition of 0.1 M NaOH solution at a maximum pump speed of 50 rpm (corresponding to a flow rate of 16 min/mL) was set up. The pH control was started 24 hours after inoculation.

| Culture conditions | |
|---|---|
| Temperature | 27°C |
| DO concentration | 50% saturation |
| pH | 6.2 |
| Rocking rate | 18–32 rpm |
| Rocking angle | 6° |
| Air flow rate (headspace) | 0.25 min/L |
| Maximum $O_2$ flow rate (headspace) | 0.25 min/L |

### Initiation of rSEAP production

The production of rSEAP was induced by infection of the Sf9 cells with the baculovirus. Virus quantification was performed using three different methods: plaque assay (PA), endpoint dilution assay (EDA), and cell growth cessation assay (CGCA), as described [1]. The effective virus titer $VT_{eff}$ was then calculated from a combination of the outcomes of these assays, as follows:

$$VT_{eff} = \frac{\left(\frac{VT_{PA} + VT_{EDA}}{2}\right) + VT_{CGCA}}{2}$$

where $VT_{PA}$, $VT_{EDA}$, and $VT_{CGCA}$ are the virus titers (in PFU/mL) from the individual virus assays. The infection parameters, multiplicity of infection (MOI) of 0.01 PFU/cell and infected cell density (ICD) of 2 x $10^6$ cells/mL, were selected based on preliminary studies performed in our laboratory. Two days after inoculation, when the live cell density in the HyPerforma Rocker BPC had reached 3.6 x $10^6$ cells/mL, the control loops were switched off and the HyPerforma Rocker BPC was disconnected from the control unit.

The infection procedure was performed under a laminar flowhood. After ensuring a live-cell density of 2 x $10^6$ cells/mL in a working volume of 5 L, the Rocker BPC was placed on scales, and 66.7 mL of the virus preparation at a dilution of 1:1,000 was added via a sterile syringe to achieve the desired MOI of 0.01 PFU/cell. The culture volume was topped with 2.35 L of fresh medium to achieve a total volume of 5 L. Afterwards, the HyPerforma Rocker BPC was placed on the rocking platform and all the controllers were switched on. The rocking rate was set to 25 rpm for 1 hour.

### Process analysis

Samples of at least 4 mL (6 mL during the production phase) were taken at least once a day from the HyPerforma Rocker BPC via the luer lock sampling port using sterile 10 mL syringes. Cell densities and viability were determined using a NucleoCounter™ NC-100™ Mammalian Cell Counter (ChemoMetec). Dilutions were performed with PBS, after the cell density reached 6 x $10^6$ cells/mL. A Cedex™ HiRes cell counting device (Roche Diagnostics) was used to 6 determine cell diameter. Nutrients and metabolites were measured using a BioProfile™ 100 Plus multi-biosensor analysis system (Labor-Systeme Flükiger AG).

The rSEAP activity was measured indirectly via enzymatic transformation of *p*-nitrophenyl phosphate into *p*-nitrophenyl at 405 nm, using a magnesium chloride buffer (magnesium chloride, homoarginine hydrochloride, and diethanolamine adjusted to pH 9.8) and *p*-nitrophenyl phosphate (disodium salt, hexahydrate) as a substrate solution. The quality of the expressed product was not investigated.

## Results

During the first 48.5 hours, the cell density in the HyPerforma Rocker BPC increased from 1 x $10^6$ cells/mL to 3.9 x $10^6$ cells/mL with a specific growth rate of 0.029 per hour, corresponding to a doubling time of 23.9 hours (Figure 2A). After infection with the baculovirus, the cells grew exponentially over the next 2 days and reached a maximum viable cell density of 6.1 x $10^6$ cells/mL. The cell viability in the HyPerforma Rocker Bioreactor was 98.9 ± 0.4% up to this point in time. The process was aborted after 163 hours postinfection (213 hours total time), when the cell viability dropped to zero.

A slightly increased cell diameter of 14.9 µm and minor rSEAP activity of about 0.1 U/mL were detected 2 days postinfection (Figure 2B). Afterwards, the rSEAP activity increased rapidly and reached 59 ± 4 U/mL at the end of the process (163 hours postinfection). This is approximately 40 hours later than in previous experiments conducted in our laboratory, despite comparable peak cell densities [2]. The reason could have been the fact that less of the virus was added, which caused fewer cells to be infected by primary infection. Hence, more infection cycles were required until all the cells in the HyPerforma Rocker BPC had been infected. The increase in rSEAP activity was accompanied by an increase in the average cell diameter to a maximum of 16.3 µm after 142 hours postinfection.

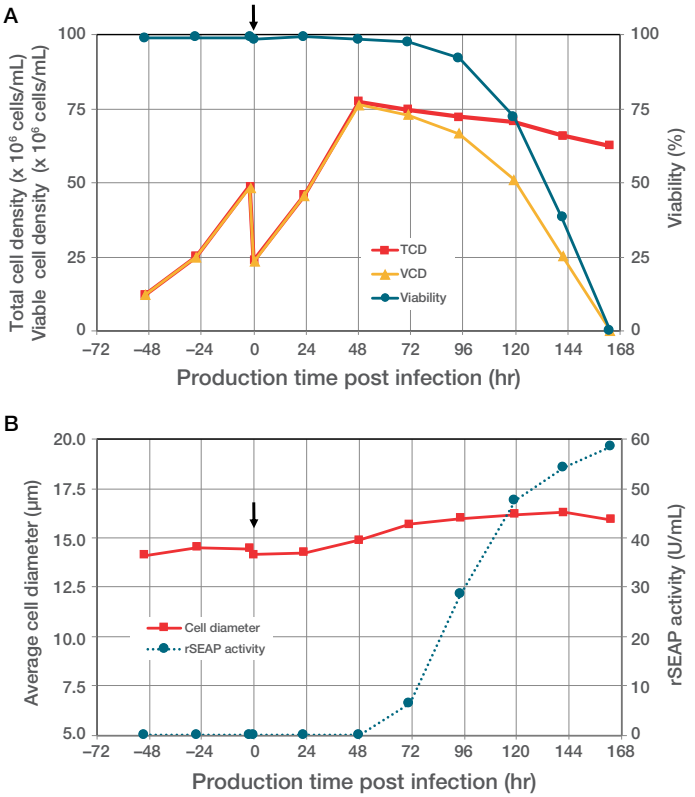


**Figure 2. Analysis of samples over the culture period.**
**(A)** Cell densities and viability. **(B)** Cell diameter and rSEAP activity. The arrow indicates the time of virus addition to initiate the protein expression.

thermo scientific

The DO concentration decreased continuously from 86% saturation after inoculation to 59% saturation before virus infection, indicating cell growth. After virus infection (indicated by the arrow in Figure 3), the rocking rate was set to a constant value of 25 rpm for 1 hour in order to ensure sufficient mixing to disperse the virus. After restarting the control loop for oxygen, the DO concentration immediately dropped to 33% saturation and the rocking rate automatically increased in order to maintain the DO concentration at the defined set point of 50% saturation. The rocking rate reached 29 rpm 57 hours postinfection, before the cells entered the stationary phase. The DO concentration in the HyPerforma Rocker BPC remained at 50.02 ± 0.14% saturation until 172 hours postinfection. No addition of pure oxygen was required.

The control loop for pH was started after 24 hours of process time and was run for 26 hours. Overall, 27 mL of 0.1 M NaOH was added. During this time, the pH in the HyPerforma Rocker BPC was 6.20 ± 0.01, indicating correct pH regulation in the HyPerforma Rocker Bioreactor. The pH values agreed well with offline measurements, with deviations below 0.2 pH units.

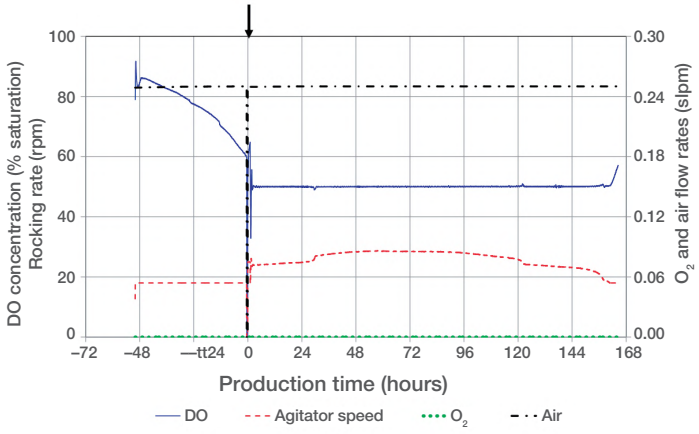


**Figure 3. Online data for DO concentration, agitation, and oxygen flow rate in the HyPerforma Rocker BPC.**

## Conclusions

The HyPerforma Rocker Bioreactor was successfully tested in a biphasic Sf9 cell–based growth and rSEAP production process. The maximum viable cell density in the HyPerforma Rocker BPC of 6.1 x $10^6$ cells/mL was measured after 48.3 hours postinfection. The maximum rSEAP activity of 59 ± 4 U/mL in the HyPerforma Rocker BPC was measured 163 hours postinfection (213 hours of process time). The results for cell growth, peak cell density, and maximum rSEAP activity are in the typical ranges observed in previous cultivation using other wave-mixed bioreactor types.

### References


1. Roldão A, Oliveira R, Carrondo MJ et al. (2009) Error assessment in recombinant baculovirus titration: evaluation of different methods. *J Virol Methods* 159(1):69–80.
2. Imseng N, Steiger N, Frasson D et al. (2014) Single-use wave-mixed versus stirred bioreactors for insect-cell/BEVS-based protein expression at benchtop scale. Engineering in Life Sciences 14(3):264–271.


### Authors


Stephan C. Kaiser, Staff Scientist, Research and Development, Thermo Fisher Scientific, Santa Clara, USA

Nadezda Perepelitsa, GMP Process Engineer, Johnson & Johnson, Basel, Switzerland


Find out more at **thermofisher.com/rockerbioreactor**

ThermoFisher SCIENTIFIC



**COL013575 1120**

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# Product Description

**Catalog Number:** 431143

**Product Description:** Corning® 125 mL disposable Erlenmeyer flask, vent cap

**Component Materials:**

| | | |
|---|---|---|
| Flask | - | Virgin Polycarbonate, meets *USP, Class VI* requirements for plastic containers and closures. |
| Cap | - | Virgin Polypropylene, meets *USP, Class VI* requirements for plastic containers and closures. Heavy metal free (meets *CONEG* req.) color concentrate.<br>0.2 μM microporous PTFE membrane, meets *USP, Class VI* requirements for plastic containers and closures. |

**Product Dimensions:**

| | | | | | |
|---|---|---|---|---|---|
| Height of flask with cap | - | 4.54 in. | Height of flask w/o cap | - | 4.45 in. |
| Diameter of flask @ widest | - | 2.58 in. | Tolerances | - | +/- 0.05 in. |
| Diameter of cap | - | 1.51 in. | | | |

**Sterilization:**
The product has been irradiated and dosimetrically released based on ANSI/AAMI/ISO 11137 *Sterilization of healthcare products-Requirements for validation and routine control-Radiation sterilization.*
Sterility Assurance Level: SAL $10^{-6}$

**Pyrogens:**

The product has been tested and has met the criteria established in the current version of ANSI/AAMI ST 72: *Bacterial Endotoxins - Test methodologies, routine testing, and alternative to batch testing.* Results: less than 0.1EU/mL.

**Bovine Spongiform Encephalopathy and Transmissible Spongiform Encephalopathy:**
This product complies with the latest revision of EMEA/410/01 "Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human veterinary medicinal products" by virtue of all bovine derived material having been processed per specific conditions of section 6.4 of EMEA/410/01.

**RNase/DNase Testing:**
This product has been tested and is free of any detectable RNase/DNase contamination.

**Performance Testing:**
Each manufacturing lot is sampled and tested in accordance with Standard Operating Procedures.

| | |
|---|---|
| Integrity Testing: | Forward pressurization of the product to 10 inches of water column. |
| Visual Attributes: | Visual examination of the product. |
| Packaging: | Inspection for seal and barrier integrity, accurate labeling, and correct product configuration. |

**Lot Number Designation:**
8 Digit Lot Number: First 3 digits - Julian date, start of manufacturing; Next 2 digits - Year of manufacture; Last 3 digits - Run number for that year.

**Revision Date:**
04-14-20

Rev No: 11

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# Product Description

**Catalog Number:** 431144

**Product Description:** Corning® 250 mL disposable Erlenmeyer flask, vent cap

**Component Materials:**

| | | |
|---|---|---|
| Flask | - | Virgin Polycarbonate, meets *USP, Class VI* requirements for plastic containers and closures. |
| Cap | - | Virgin Polypropylene, meets *USP, Class VI* requirements for plastic containers and closures. Heavy metal free (meets *CONEG* req.) color concentrate. 0.2 μM microporous PTFE membrane, meets *USP, Class VI* requirements for plastic containers and closures. |

**Product Dimensions:**

| | | | | | |
|---|---|---|---|---|---|
| Height of flask with cap | - | 5.50 in. | Height of flask w/o cap | - | 5.42 in. |
| Diameter of flask @ widest point | - | 3.28 in. | Tolerances | - | +/- 0.05 in. |
| Diameter of cap | - | 1.69 in. | | | |

**Sterilization:**
The product has been irradiated and dosimetrically released based on ANSI/AAMI/ISO 11137 *Sterilization of healthcare products-Requirements for validation and routine control-Radiation sterilization.*
Sterility Assurance Level: SAL $10^{-6}$

**Pyrogens:**

The product has been tested and has met the criteria established in the current version of ANSI/AAMI ST 72: *Bacterial Endotoxins - Test methodologies, routine testing, and alternative to batch testing.* Results: less than 0.1EU/mL.

**RNase/DNase Testing:**
This product has been tested and is free of any detectable RNase/DNase contamination.

**Bovine Spongiform Encephalopathy and Transmissible Spongiform Encephalopathy:**
This product complies with the latest revision of EMEA/410/01 "Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human veterinary medicinal products" by virtue of all bovine derived material having been processed per specific conditions of section 6.4 of EMEA/410/01.

**Performance Testing:**
Each manufacturing lot is sampled and tested in accordance with Standard Operating Procedures.

| | |
|---|---|
| Integrity Testing: | Forward pressurization of the product to 10 inches of water column. |
| Visual Attributes: | Visual examination of the product. |
| Packaging: | Inspection for seal and barrier integrity, accurate labeling, and correct product configuration. |

**Lot Number Designation:**
8 Digit Lot Number: First 3 digits - Julian date, start of manufacturing; Next 2 digits - Year of manufacture; Last 3 digits - Run number for that year.

**Revision Date:**
04-14-20

Rev No: 12

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# Product Description

**Catalog Number:** 431145

**Product Description:** Corning® 500 mL disposable Erlenmeyer flask, vent cap

**Component Materials:**

| | | |
|---|---|---|
| Flask | - | Virgin Polycarbonate, meets *USP, Class VI* requirements for plastic containers and closures. |
| Cap | - | Virgin Polypropylene, meets *USP, Class VI* requirements for plastic containers and closures. Heavy metal free (meets *CONEG* req.) color concentrate. 0.2 μM microporous PTFE membrane, meets *USP, Class VI* requirements for plastic containers and closures. |

**Product Dimensions:**

| | | | | | |
|---|---|---|---|---|---|
| Height of flask with cap | - | 7.05 in. | Height of flask w/o cap | - | 6.96 in. |
| Diameter of flask @ widest point | - | 3.99 in. | Tolerances | - | +/- 0.05 in. |
| Diameter of cap | - | 1.91 in. | | | |

**Sterilization:**
The product has been irradiated and dosimetrically released based on ANSI/AAMI/ISO 11137 *Sterilization of healthcare products-Requirements for validation and routine control-Radiation sterilization.*
Sterility Assurance Level: SAL $10^{-6}$

**Pyrogens:**

The product has been tested and has met the criteria established in the current version of ANSI/AAMI ST 72: *Bacterial Endotoxins - Test methodologies, routine testing, and alternative to batch testing.* Results: less than 0.1EU/mL.

**RNase/DNase Testing:**
This product has been tested and is free of any detectable RNase/DNase contamination.

**Bovine Spongiform Encephalopathy and Transmissible Spongiform Encephalopathy:**
This product complies with the latest revision of EMEA/410/01 "Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human veterinary medicinal products" by virtue of all bovine derived material having been processed per specific conditions of section 6.4 of EMEA/410/01.

**Performance Testing:**
Each manufacturing lot is sampled and tested in accordance with Standard Operating Procedures.

| | |
|---|---|
| Integrity Testing: | Forward pressurization of the product to 10 inches of water column. |
| Visual Attributes: | Visual examination of the product. |
| Packaging: | Inspection for seal and barrier integrity, accurate labeling, and correct product configuration. |

**Lot Number Designation:**
8 Digit Lot Number: First 3 digits - Julian date, start of manufacturing; Next 2 digits - Year of manufacture; Last 3 digits - Run number for that year.

**Revision Date:**
04-14-20

Rev No: 12

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# Product Description

**Catalog Number:** 431685

**Product Description:** Corning® 5 Liter disposable Erlenmeyer Flask, easy grip vent cap with liner

**Component Materials:**

| | | |
|---|---|---|
| Flask | - | Virgin Polycarbonate, meets *USP, Class VI* requirements for plastic containers and closures. |
| Vent Cap | - | Virgin Polypropylene, meets *USP, Class VI* requirements for plastic containers and closures. Heavy metal free (meets *CONEG* req.) color concentrate. 0.2 μM microporous PTFE membrane, meets *USP, Class VI* requirements for plastic containers and closures. Thermoplastic elastomer liner, meets *USP, Class VI* requirements for plastic containers and closures. |

**Product Dimensions:**

| | | | | | |
|---|---|---|---|---|---|
| Height of flask with cap | - | 11.33 in. | Diameter of cap (OD) | - | 4.16 in. |
| Height of flask w/o cap | - | 11.24 in. | Diameter of neck (OD) | - | 3.88 in. |
| Diameter of flask @ widest point | - | 9.05 in. | Tolerances | - | +/- 0.05 in. |

**Sterilization:**
The product has been irradiated and dosimetrically released based on ANSI/AAMI/ISO 11137 (TIR 33) *Sterilization of healthcare products-Requirements for validation and routine control-Radiation sterilization.* Sterility Assurance Level: SAL $10^{-6}$

**Pyrogens:**
The product has been tested and has met the criteria established in the current version of ANSI/AAMI ST 72: *Bacterial Endotoxins - Test methodologies, routine testing, and alternative to batch testing.* Results: less than 0.1EU/mL

**RNase/DNase Testing:**
This product has been tested and is free of any detectable RNase/DNase contamination.

**Bovine Spongiform Encephalopathy and Transmissible Spongiform Encephalopathy:**
This product complies with the latest revision of EMEA/410/01 "Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human veterinary medicinal products" by virtue of all bovine derived material having been processed per specific conditions of section 6.4 of EMEA/410/01.

**Performance Testing:**
Each manufacturing lot is sampled and tested in accordance with Standard Operating Procedures.

| | |
|---|---|
| Integrity Testing: | Inverted leak test with an alcohol based solution. |
| Visual Attributes: | Visual examination of the product. |
| Packaging: | Inspection for seal and barrier integrity, accurate labeling, and correct product configuration. |

**Lot Number Designation:**
8 Digit Lot Number: First 3 digits - Julian date, start of manufacturing; Next 2 digits - Year of manufacture; Last 3 digits - Run number for that year.

**Revision Date:**
04-14-20

Rev No: 2

# **WAVE Bioreactor** Systems 2/10 and 20/50

CELL PREPARATION

The WAVE Bioreactor™ system is a cell culture platform suitable for applications in gene and cell therapy and regenerative medicine. Culture medium and cells only come into contact with a presterilized, disposable chamber known as the Cellbag™ bioreactor, which is placed on a rocking platform (Fig 1). The rocking motion of the platform induces waves to mix and transfer oxygen to the culture medium to create an optimal environment for cell growth (Fig 2). As a result, a single Cellbag bioreactor can be used to produce cell concentrations that are suitable for clinical trials (1, 2, 3).

Key performance characteristics are:

- Functionally closed system for single-use: no cleaning, cross-contamination, and minimal validation. Cells only come into contact with disposable sterile biocompatible plastics.
- The system is suitable for cGMP production without the use of an incubator and can be easily connected to other closed cell processing devices by sterile welding
- Cellbag bioreactors that include all fittings and filters are supplied sterile and ready for use
- Allows rapid production of up to $10^7$ viable cells per mL of culture
- Multiple instrument configurations for suspension, microcarrier, batch, fed-batch, or perfusion culture
- Full scalability — the featured systems are capable of handling culture volumes from 200 mL to 25 L

## A new way of working

There is a considerable difference between using Cellbag bioreactors and conventional bags. The gentle wave action limits shear stress on the cells in comparison to traditional stirred tank bioreactors. For volumes up to 25 L, only one Cellbag bioreactor is needed. This makes the system easy to use and considerably less laborious than using static cultures where multiple bags or flasks are often needed. The perfusion culture functionality allows for automated feeding of cultures and maximizes the removal of waste products while keeping culture volumes to a minimum.

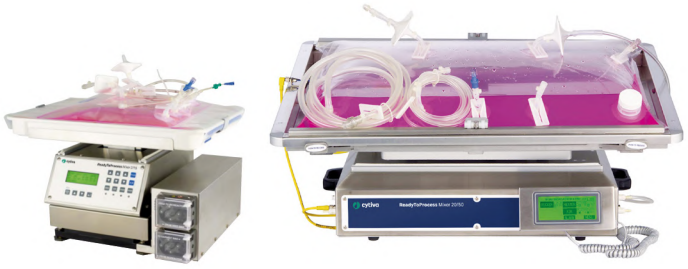

**Fig 1.** WAVE Bioreactor Systems 2/10 (left) and 20/50 (right).

The system minimizes contamination risk and makes it suitable for cGMP production. The ability to automatically monitor and control multiple parameters also contributes to low contamination risks, shorter processing time and low costs.

Systems 2/10 and 20/50 can deliver key economic benefits. Since cell cultures can reach target concentrations in one Cellbag bioreactor, the costs of disposables can be a fraction of those incurred when using other methods. The Cellbag bioreactor is easy to customize and allows the user to incorporate the WAVE Bioreactor system into their current workflow.

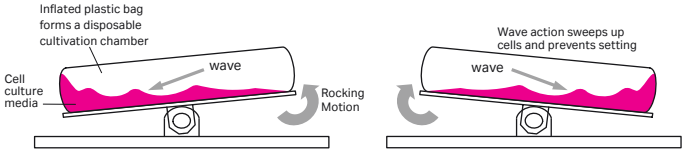


**Fig 2.** Wave action sweeps up cells and prevents settling.

# System selection

To select the correct WAVE Bioreactor system for your application, first determine the Cellbag bioreactor size you need by estimating the culture volume you require and then select the appropriate instrumentation and options (see Table 1).

**Table 1.** Specifications for WAVE Bioreactor Systems 2/10 and 20/50

| | BASE 2/10EH | BASE 20/50EHT |
|---|---|---|
| Working volume (L) | 0.2–5 | 0.2–25 |
| Integral features | Speed/Angle control<br>Temperature control<br>Aeration | Speed/Angle control<br>Temperature control<br>Aeration |
| Options | CO2MIX<br>O2MIX<br>DOOPT20<br>pH<br>PERFUSION CTRL | WAVEPOD<br>CO2MIX<br>O2MIX<br>DOOPT20<br>pH<br>LOADCELL<br>AIR/TEMP DUAL<br>KIT20EHTD<br>KIT50EHTD |
| Weight | 4.2 kg (9 lbs) | 15.5 kg (34 lbs) |
| Dimensions of base | 230 × 330 × 160 mm | 573 × 465 × 179 mm |
| Dimensions with kits | KIT2EH<br>489 × 330 × 200 mm | KIT20EHT<br>711 × 575 × 254 mm<br>KIT50EHT<br>775 × 700 × 254 mm |
| Utilities | 110/230 VAC | 110/230 VAC<br>6/3 A 50/60 Hz |

WAVE Bioreactor 2/10EH is designed for quick set-up and can be used with working culture volumes of between 200 mL and 5 L. This compact unit is fitted with integral features such as aeration, heating, and temperature control. The System 2/10EH has an integral air pump with mass flow meter and is equipped with temperature control (heater and sensor). Other options include weight controllers for perfusion culture, dissolved oxygen amplifiers, and pH controllers. The data acquisition software product PCDAQ/S is available for recording temperature, rocking speed/angle and weight.

WAVE Bioreactor System 20/50 is intended for working culture volumes between 200 mL and 25 L. This system is very versatile and is suitable for research and development as well as for production use. The System 20/50 is modular, consisting of a base unit that is electrically powered and a kit for holding the Cellbag bioreactor. System 20/50 has the following options: KIT20EHT for up to 10 L of culture or KIT50EHT for up to 25 L culture volume.

Other options include weight controllers for perfusion culture, dissolved oxygen amplifiers, and pH controllers. For added convenience the WAVE 20/50 system can be used with the WAVEPOD™ Controller (Fig 3) that allows simplified control of all culture parameters including oxygen, pH, and $CO_2/O_2$ gas mixing in a single module. The System 20/50 can also be configured to allow independent control of two individual cultures using a single base unit.


**Fig 3.** WAVEPOD Integrated controllers are available with left- or right-facing probe connections for ease of use.

# Bioreactor bags

Cellbag bioreactors (Fig 4) are intended for single-use and are suitable for cGMP production of human cells for cell therapy applications. The components are similar to those used for biological storage bags and meet USP Class VI specifications for plastics.

Cellbag bioreactors are manufactured from multilayer laminated clear plastic films designed to provide high mechanical strength and bioinert fluid contact. Typically, the fluid contact layer is a medical grade, low density polyethylene. The second or outer layer provides mechanical strength and a gas-impermeable barrier. This non-contact layer is typically made of low density polyethylene, EVA, or Nylon/EVOH copolymers.

Cellbag bioreactors are sterilized by gamma radiation at 25–40 kGy. Individual radiation indicators are affixed to each Cellbag bioreactor. Each lot is also tested for bacterial endotoxin levels. Lot release requires an endotoxin level below 0.125 EU/mL.

Validation data and Cellbag Drug Master File data are available to demonstrate biocompatibility; the user is however advised to validate suitability for specific applications.

Cellbag bioreactors can be used from 0°C to 50°C. Each chamber is tested for leakage by a pressure-hold test prior to sterilization (maximum operating pressure is 1.5 psig [0.1 bar]).

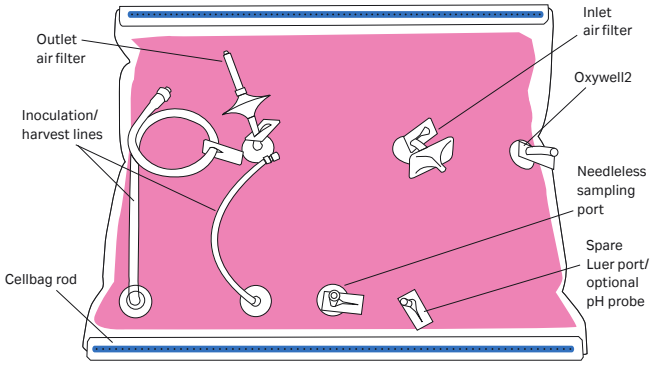


**Fig 4.** The Cellbag bioreactor can be fitted with a number of optional features.

### *References*


1. Hollyman, D. *et al*. Manufacturing Validation of Biologically Functional T Cells Targeted to CD19 Antigen for Autologous Adoptive Cell Therapy. *J. Immunother.* **32**, 169–180 (2009).
2. Tran, C-A. *et al*. Manufacturing of Large Numbers of Patient-specific T Cells for Adoptive Immunotherapy. *J. Immunother.* **30**, 644-654 (2007).
3. Sadeghi, A. *et al.* Large-scale bioreactor expansion of tumor-infiltrating lymphocytes. *J. Immunol. Meth.* **364**, 94-100 (2011).


# Ordering information

### *WAVE Bioreactor System 2/10*

| Description | Quantity | Code No |
|---|---|---|
| BASE 2/10EH | 1 | 28-9377-86 |
| Perfusion Controller with load cell | 1 | 28-9884-64 |
| CO2MIX 20 | 1 | 28-9377-95 |
| Protective Lid, allows use of system with light-sensitive cells | 1 | 28-9376-33 |
| Cellbag 200 mL to 1 L with internal perfusion filter and Oxywell2 | 1 | 28-9376-52 |
| Cellbag 500 mL to 5 L with internal perfusion filter and Oxywell2 | 1 | 28-9376-62 |
| PCDAQ/S | 1 | 28-4116-49 |

### *WAVE Bioreactor System 20/50*

| Description | Quantity | Code No |
|---|---|---|
| BASE 20/50EHT-CO2-L, 100-120V | 1 | 28-9436-96 |
| BASE 20/50EHT-CO2-L, 220-240V | 1 | 28-9436-97 |
| KIT20EHT, 100-120V | 1 | 28-9376-34 |
| KIT20EHT, 220-240V | 1 | 28-9481-58 |
| KIT50EHT, 100-120V | 1 | 28-9384-26 |
| KIT50EHT, 220-240V | 1 | 28-9481-65 |
| Protective Lid, allows use of 20 EHT Kit with light-sensitive cells | 1 | 28-9376-35 |
| Peristaltic Feed/Harvest Pump | 1 | 28-9376-51 |
| Cellbag 500 mL to 5 L with internal perfusion filter and Oxywell2 | 1 | 28-9376-62 |
| Cellbag 1 L to 10 L with internal perfusion filter and Oxywell2 | 1 | 28-9376-64 |

### *Cellbag bioreactors*

| Description | Quantity | Code No |
|---|---|---|
| Cellbag 200 mL to 1 L, Basic | 1 | 28-9378-00 |
| Cellbag 500 mL to 5 L, Basic | 1 | 28-9378-01 |
| Cellbag 1 L to 10 L, Basic | 1 | 28-9378-02 |
| Cellbag 5 L to 25 L, Basic | 1 | 28-9376-66 |
| Cellbag 200 mL to 1 L with internal perfusion filter and Oxywell2 | 1 | 28-9376-52 |
| Cellbag 500 mL to 5 L with internal perfusion filter and Oxywell2 | 1 | 28-9376-62 |
| Cellbag 1 L to 10 L with internal perfusion filter and Oxywell2 | 1 | 28-9376-64 |
| Cellbag 5 L to 25 L with internal perfusion filter and Oxywell2 | 1 | 28-9376-68 |

### *Related literature*

| | Code No |
|---|---|
| Rapid production of clinical grade T lymphocytes in the WAVE Bioreactor. Case study | 28-9331-49 |
| Perfusion culture of T lymphocytes in the WAVE Bioreactor System 2/10. Application note | 28-9650-52 |
| Perfusion culture of human natural killer cells in the WAVE Bioreactor 2/10 system. Application note | 28-9936-25 |

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GE Healthcare
Life Sciences

Data file 29-0237-13 AA

ReadyToProcess™

# WAVE Bioreactor™ 200 system

WAVE Bioreactor 200 system (Fig 1) is part of GE Healthcare Life Sciences' ReadyToProcess platform of ready-to-use products. The system is a cell culture device for the production of recombinant proteins in mammalian and insect cell lines in batch, fed-batch, and perfusion culture. Culture medium and cells are loaded into a single-use, presterilized bag known as the Cellbag™ bioreactor. The Cellbag bioreactor is placed on an electric rocking base. The rocking motion of this base induces waves in the cell culture fluid within the Cellbag bioreactor to provide efficient mixing and gas transfer. The resulting environment within the Cellbag bioreactor can easily support $1 \times 10^7$ cells/mL. The Cellbag bioreactor requires no cleaning or sterilization, providing easy operation and protection against cross-contamination.

As part of ReadyToProcess platform, the WAVE Bioreactor brings flexibility and speed to upstream and downstream processing of biologicals. The product range comprises WAVE Bioreactor systems, WAVE Mixer™, tubing sealers and fusers, hollow fiber and normal flow filters, prepacked chromatography columns, and ÄKTA™ ready chromatography system with a disposable flow path, as well as the assemblies and connections in between. The platform is scalable from the lab bench to manufacturing.

### WAVE Bioreactor 200 system delivers:

- Convenience: Presterilized, single-use Cellbag bioreactors protect against the risk of cross-contamination, require no cleaning, and involve minimal validation and they are supplied in a ready-to-use format
- Reliability: Cellbag bioreactors, including all fittings and filters, are supplied sterile and ready for use. They are suitable for cGMP commercial production and a biosafety cabinet is not required for inoculation or sampling
- Flexibility: Multiple instrument configurations for suspension, microcarrier, batch, fed-batch, or perfusion culture
- Versatility: The 200 system is capable of handling culture volumes from 10 to 100 L

**Fig 1.** The WAVE Bioreactor 200 system is suitable for culture volumes of 10 to 100 L.

## System descriptions

The WAVE Bioreactor 200 system comprises integral rocking, temperature, weight, and airflow controllers. The self-contained system is designed for use with working culture volumes of 10 to 100 L in applications such as inoculum scale-up, R & D, commercial production, vaccine production, and antibody manufacture. For additional flexibility, optional modules including DO, $CO_2$, $O_2$, weight/perfusion, and pH control can be added while builtin Ethernet and MODBUS data ports allow communication with other software.

## Applications

The WAVE Bioreactor system is suitable for use with anchorage-dependent cells in addition to cell suspensions and has applications in:

- Monoclonal antibodies
- Insect cell culture
- Virus production
- Growing pathogens or other high-containment systems
- Inoculum scale-up
- Protein expression
- Primary cell line expansion

# Components

## Touchscreen

The color touchscreen (Fig 2) located on the control panel of the WAVE Bioreactor 200 system enables the setup, control, and viewing of all cell culture parameters while data can be monitored graphically in real time. The main menu provides an overview of key operating conditions and it is the main access screen for all controls. Different control buttons are displayed depending on the options enabled. Pressing the desired button will take you to the respective control screen. The touchscreen is housed in a stainless steel enclosure and can be tilted and rotated for easier viewing.

**Fig 2.** The touchscreen provides easy access to all control functions.

## Expansion slots

Optional modules such as the dissolved oxygen (DO), $O_2$, $CO_2$, pH, and dual system controllers can be added to standard WAVE Bioreactor systems to monitor and control additional parameters as your requirements change. These modules plug into the front and back instrument panels (Fig 3) of the base unit and are enabled via the instrument's configuration and calibration functions in order to display the module variables and controls on the color touchscreen. Spare racks are included for future instrument options.


**Fig 3.** Expansion slots are provided for the installation of optional instrumentation.

## Linear electronic motor

An electric linear motor is used to rock the base units of the WAVE Bioreactor 200 system. Unlike geared motors, this electromagnetic device has only one moving part and provides greater reliability. The linear motor follows a preset and optimal speed and acceleration profile to provide the most effective wave for efficient low-shear mixing.

## UNICORN™ DAQ

UNICORN DAQ 1.0 software facilitates real time data acquisition for the management and evaluation of results from cell cultures performed using up to four different WAVE Bioreactor systems connected to a single PC. The WAVE Bioreactor system can be connected directly or networked to the software providing a common platform and user interface for monitoring and storing result data. A dynamic graphical user interface informs you about the real-time status of the run being monitored. During a run, data is automatically saved to a local hard drive or server in a secure and unalterable result file for added security.

## Quick-release bag holder

Rapid release Cam-lock levers secure the Cellbag bioreactor in place on the rocking platform allowing bags to be attached and removed in minutes. The holder design ensures that the Cellbag bioreactor is locked in the optimal position for oxygen transfer and mixing.

## Stainless steel construction

The stainless steel housing of WAVE Bioreactor 200 system completely encloses the disposable Cellbag bioreactor and protects it from accidental damage. The housing is capable of containing potential spills. The WAVE Bioreactor 200 system is mounted on casters for mobility.

# Optional components

## pH monitor

The pH monitor provides amplification, display, and data transmission of pH allowing real-time measurement of pH in the Cellbag Bioreactor. The pH monitor was designed for use with pH sensor integrated into the WAVE Cellbag

## Dissolved oxygen monitor

The DO monitor provides amplification, display, and data transmission of DO concentration allowing real-time measurement of DO concentration inside the Cellbag bioreactor. The DO monitor controller was designed for use with DO sensor integrated into the WAVE Cellbag, and it can increase the rocking rate or gas concentration automatically to maintain online control of DO.

## $O_2$/air mix controller

The $O_2$/air mix controller connects to a supply of oxygen (and low pressure $N_2$ supply if required) to provide $O_2$/air concentrations between 0% and 50% $O_2$. The instrument controls enriched oxygen levels for insect cell/baculovirus and high culture density applications; it is also useful for maintaining low-oxygen environments for near-anaerobic applications.

### $CO_2$/air mix controller

The $CO_2$/air mix controller connects to a supply of 100% $CO_2$ to provide $CO_2$/air concentrations between 0% and 15% $CO_2$. The instrument is useful for pH control of bicarbonate buffered cell culture media.

### Temperature control

Temperature is regulated for single and dual WAVE Bioreactor systems (Fig 4).

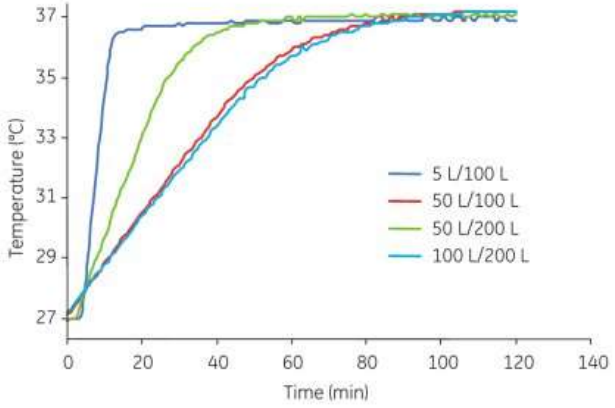



**Fig 4.** Temperature control from 27°C to 37°C in 5 L, 50 L in 100 L bag, 50 L, 100 L in 200 L bag.

### Dual Cellbag control system

The WAVE Bioreactor 200 system can be configured for either single or dual Cellbag bioreactor control. In the dual-bag configuration, the menu on the touchscreen would display left- and right-bag values for certain parameters such as bag pressure and temperature. In the single-bag configuration, only data for the left-hand side bag is shown. Instruments configured as dual bag systems can be set to single-bag operation using the setup screen.

### Analog output card

Up to eight channels of analog outputs are available as an option for controlling instrument variables such as rocking speed, weight, airflow, temperature, pH, DO, $CO_2$, and $O_2$ within their preset ranges. The DB25-pin analog output connector is located on the rear panel of the rocker base unit. Two analog output cards are required for dual-configured systems.

### Loadcell

Electronic loadcell modules provide online measurement of Cellbag bioreactor weight and can be used for automated filling and harvesting of media. A built-in pump controller maintains a constant volume for perfusion operations. Loadcell modules are optional factory-installed accessories for WAVE Bioreactor 200 system.

## Technical information and specifications

**WAVE Bioreactor 200 system**

| | |
|---|---|
| Features | Touch panel operator interface |
| | Direct drive electronic linear motor |
| | Adjustable rocking rate from 4 to 25 rocks/min with acceleration control |
| | Adjustable rocking angle from 2° to 9° Integral temperature controller with heater Integral weight controller |
| | Integral airflow controller |
| | Integral PID controller for automatic temperature, $O_2$, $CO_2$, DO, and pH adjustment |
| | Real-time data monitoring |
| | RS-485 MODBUS communications port |
| | 10Base-T Ethernet communications port Remote alarm contact and printer interface Stainless-steel containment enclosure |
| Dimensions (L × W × H) | Base unit: 1852 × 1096 × 1120 mm (73 × 43 × 44 in) |
| Weight (empty) | 350 kg (780 lb) |
| Utilities | Voltage: 100-120/220-240 VAC |
| | Frequency: 50/60 Hz |
| | Maximum current: 15 A |
| | Power: 12 KVA |
| Environmental | This equipment is designed for use under the following conditions: |
| | • Indoor use |
| | • 5°C to 40°C |
| | • Up to 80% maximum relative humidity (rh) at 31°C decreasing linearly to 50% rh at 40°C |

## Ordering information

| Product | Code number |
|---|---|
| SYSTEM200EH,CO2 | 28-4115-57 |
| SYSTEM200EH,CO2,O2 | 28-4115-55 |
| SYSTEM200EH, CO2,O2,AN | 28-4115-52 |
| SYSTEM200EH CO2 PHOPT AN | 28-9849-54 |
| SYSTEM200EH O2 DOOPT II AN | 29-0042-81 |
| SYSTEM200EH CO2 O2 DOOPT II PHOPT AN | 29-0016-41 |
| SYSTEM200EHD | 28-9366-86 |
| SYSTEM200EHD,CO2,AN | 28-4115-45 |
| SYSTEM200EHD,CO2,O2,AN | 28-4115-44 |
| SYSTEM200EHD CO2 PHOPT AN | 28-9849-53 |
| SYSTEM200EHD O2 DOOPT II AN | 29-0042-82 |
| SYSTEM200EHD CO2 O2 DOOPT II PHOPT AN | 29-0016-37 |

### Related literature

| | |
|---|---|
| Disposable Cellbag bioreactors for the WAVE Bioreactor system, Data file | 28-9511-36 |
| ReadyToProcess connectivity, Data file | 29-0138-84 |
| WAVEPOD™ II Integrated Controller, Data file | 28-9606-57 |
| WAVE Bioreactor 2/10 and 20/50 systems, Data file | 28-9520-58 |
| WAVE Bioreactor 500/1000 system, Data file | 29-0237-12 |

For local office contact information, visit
**www.gelifesciences.com/contact**

www.gelifesciences.com/readytoprocess

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work



GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

29-0237-13 AA 06/2012

# HyPerforma 2:1 500 L Single-Use Bioreactor

## The next generation of performance

The Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) provides enhanced functionality, ease of use, and efficiency. The complete HyPerforma S.U.B. system consists of a bioreactor tank and Thermo Scientific™ HyPerforma™ S.U.B. BioProcess Container (BPC), which is available in 50, 100, 250, 500, 1,000, and 2,000 L sizes with a 2:1 turndown ratio. The redesigned HyPerforma S.U.B. maintains traditional stirred-tank bioreactor design principles including specific height-to-diameter ratios and optimized mixer location that deliver optimum cell viability, performance, and scalability from process development through production.

This data sheet provides information on the 500 L S.U.B. system, which includes the tank and standard S.U.B. BPC. The BPC utilizes dual-sparger configurations with a porous-frit sparger and drilled-hole or open-pipe sparger that have been rigorously tested to provide high $k_La$ values and optimal $CO_2$ stripping for improved pH control and decreased foaming.

## The S.U.B. system consists of the following components:

### S.U.B. hardware unit—available in turnkey format

- Complete mixing system with water-jacketed vessel
- Drive shaft inserts into the S.U.B. BPC through the mixing drive motor and locks into the BPC agitator assembly

### S.U.B. BPC—supplied sterile and ready to use

- Agitator assembly is a single-use (polyethylene) impeller with a bearing-and-seal assembly linked to an external mixer drive
- Dual gas spargers available with either drilled-hole or open-pipe sparger and standard porous-frit sparger
- Vent filter outlet for system exhaust

- Integrally sealed ports in the S.U.B. BPC allow for addition of sensor probes and line sets
- Available in Thermo Scientific™ CX5-14 Film and Thermo Scientific™ Aegis™ 5-14 Film options

### System options—adaptable to your needs

- Optional electrical box for remote agitation control
  - Water-jacketed S.U.B.s require a separate external temperature control unit
- Exhaust gas vent filter heaters
- Load cells (standard on 1,000 and 2,000 L S.U.B.s)
- Cable management tree
- Process control system
- See Table 12 for auxiliary components for S.U.B. control management; choose an open-architecture approach or a turnkey, ready-to-use Thermo Scientific S.U.B. system

ThermoFisher SCIENTIFIC

## Standard S.U.B. hardware units

The 500 L standard S.U.B. hardware units are available in the following configurations (Table 1).

- Water jacket with DC motor
- Water jacket with AC motor

Additional options are listed in Tables 3–7.

**Table 1. 500 L standard S.U.B. hardware unit with casters (leveling feet).**

| Description | Cat. No. |
|---|---|
| Water jacketed, DC motor | SUB0500.9002 |
| Water jacketed, 240 VAC, AC motor | SUB0500.9006 |

## Design features

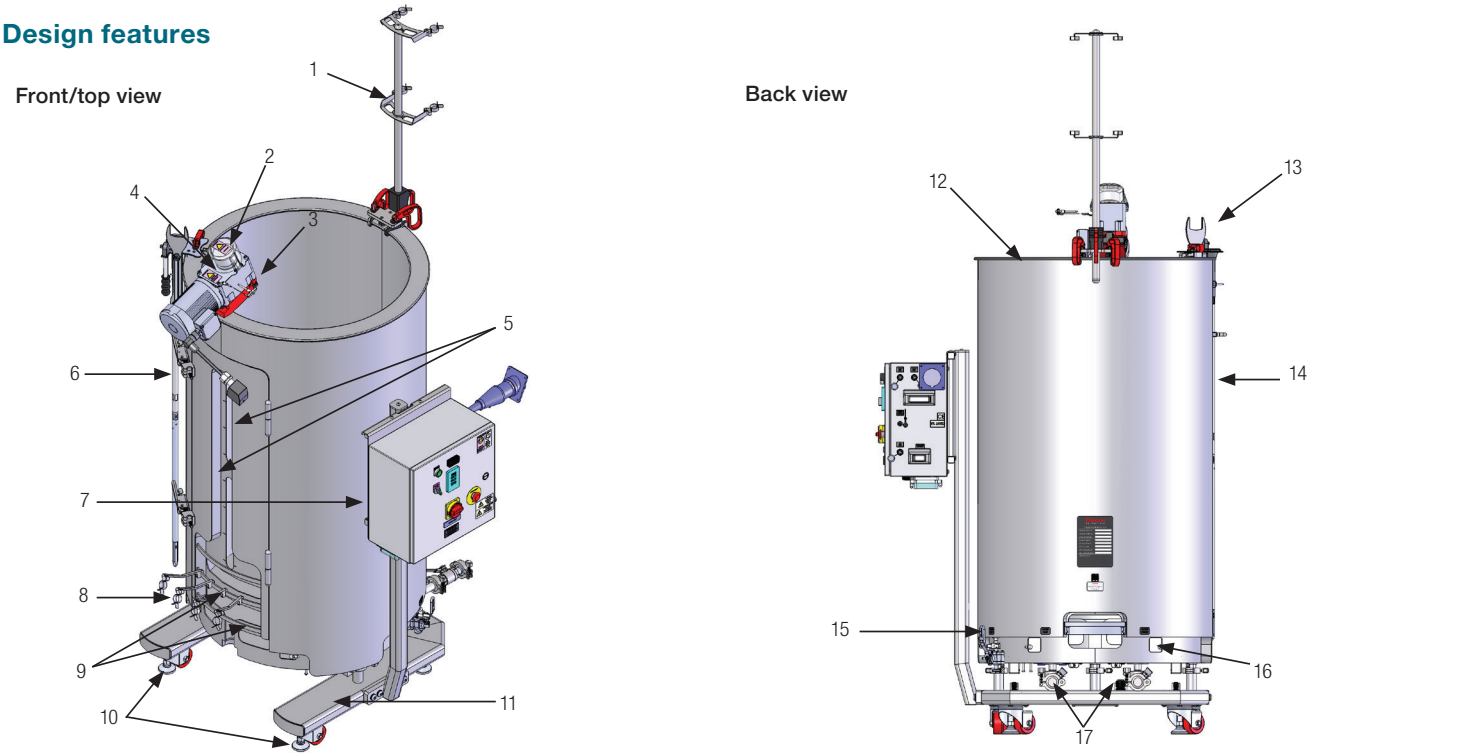
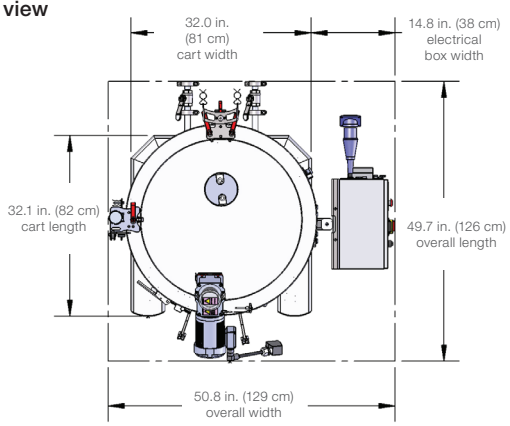



**Figure 1. 500 L S.U.B. hardware unit with water jacket.**

1. Exhaust vent filter holder
2. Mixing assembly with shield
3. Bearing port receiver with clamp
4. Mixer motor
5. Liquid sight windows
6. Drive shaft (stored)
7. Electrical control panel (optional)
8. Probe hanger bracket
9. Probe access windows
10. Leveling casters
11. Cart assembly
12. 3/8 in. dimpled water jacket
13. Standard tool set: 3/8 in. x 150 in.-lb square torque wrench; load cell and motor cap lockout wrench
14. Stainless steel outer support container
15. Bleed valve
16. Bottom cutouts/pins for BPC attachment and alignment
17. Quick-connect water inlet/outlet ports

**Note:** Load cells are standard only on 1,000 and 2,000 L S.U.B. hardware units.

Top view




Front view

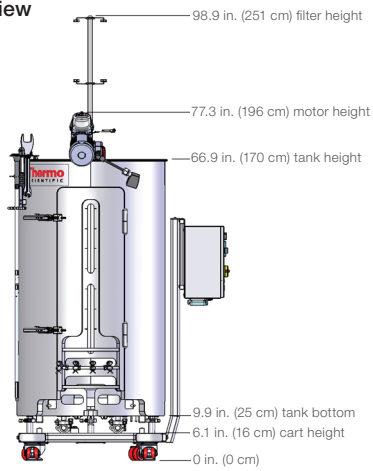



**Figure 2. 500 L S.U.B. hardware unit dimensions.**

**Table 2. 500 L S.U.B. system specifications.**

| Specifications for water-jacketed systems with AC and DC motors | | | |
|---|---|---|---|
| | | AC motor | DC motor |
| Bioreactor geometry | Rated liquid working volume | 500 L | |
| | Minimum liquid working volume | 250 L | |
| | Total bioreactor volume (liquid and gas) | 660 L | |
| | BPC chamber diameter | 75.6 cm (29.8 in.) | |
| | BPC chamber shoulder height | 152.4 cm (60.0 in.) | |
| | Liquid height at rated working volume | 113.4 cm (44.6 in.) | |
| | Fluid geometry at working volume (height:diameter ratio) | 1.5:1 | |
| | Overall bioreactor geometry (height:diameter ratio) | 1.9:1 | |
| | Tank baffles | No | |
| Impeller | Impeller (quantity x blade count) | 1 x 3 | |
| | Impeller scaling (impeller diameter/tank diameter) | 1/3 | |
| | Impeller blade pitch (angle) | 45° | |
| | Impeller diameter | 25.1 cm (9.9 in.) | |
| | Impeller calculated power number (N) | 2.1 | |
| Agitation | Maximum mixing rate | 30–150 rpm | |
| | Nominal agitation rating (power/volume) | 20 W/m$^3$ | |
| | Nominal agitation | 50% working volume: 80 rpm<br>100% working volume: 101 rpm | |
| | Nominal tip speed | 137.2 cm/s (270 ft/min) | |
| | Counterclockwise mixing flow direction | Down-pumping | |
| | Agitation shaft resolved angle | 19.6° | |
| | Agitation shaft centerline offset | 5.1 cm (2.0 in.) | |
| | Overall drive shaft length | 127.0 cm (50.0 in.) | |
| | Drive shaft diameter | 1.9 cm (0.75 in.) | |
| | Drive shaft poly-sheath outside diameter | 3.5 cm (1.4 in.) | |
| | Impeller clearance from tank bottom | 25.1 cm (9.9 in.) | |

Table 2. 500 L S.U.B. system specifications (continued).

| Specifications for water-jacketed systems with AC and DC motors | | | | |
|---|---|---|---|---|
| | | | AC motor | DC motor |
| Motor | Agitation motor drive (type, voltage, phase), AC motor only | | Induction, 208 VAC, 3 | – |
| | Agitation motor drive (type, voltage), DC motor only | | – | Brushless, 48 VDC |
| | Motor power rating (AC motor) | | 0.5 hp (372.8 W) | – |
| | Motor power rating (DC motor) | | – | 0.536 hp (400 W) |
| | Motor torque rating | | 9.5 N-m (82 in.-lb) | – |
| | Gear reduction | | 10:1 | |
| | Programmable VFD, remote panel interface, power fault auto restart | | Standard | – |
| | Motor communication methods (for external controller) | | 0–10 V, 4–20 mA, ModBus | – |
| Temperature control | Water jacket | Jacket area: full/half volume | 2.0/0.8 $m^2$ (21.4/8.4 $ft^2$) | |
| | | Jacket volume | 15.2 L | |
| | | Jacket flow rate at 50 psi (3.4 bar) | 136 L/min | |
| | | Process connection | 1 in. sanitary tri-clamp | |
| | | Nominal heating/cooling load (W) | 6.7 hp (5,000 W) | |
| | | Approximate liquid heat-up time (5–37°C) | 2.7 hr | |
| | Misc. | RTD or thermocouple, 1/8 in. (3.2 mm) OD | RTD: Pt-100 (standard) | |
| Support container | Overall width | | 125.2 cm (49.3 in.) with E-Box | 86.4 cm (34.0 in.) |
| | Overall length | | 124.4 cm (47.8 in.) with E-Box | 116 cm (45.7 in.) |
| | Overall height | | 251 cm (99 in.) | |
| | Dry skid weight (mass) | | 354 kg (780 lb) | |
| | Wet skid weight, rated working volume (mass) | | 854 kg (1,882 lb) | |
| General | Ceiling height required for drive shaft loading | | 267 cm (105 in.) | |
| | Electrical power supply requirement (voltage, phase, current) | | 208–240 VAC, single, 10 A | Dependent on controller |
| | Tested system reliability (minimum) | | 0.9 at 90% | |
| | pH and DO probe, autoclavable type (Applisens™, Broadley James™, Mettler Toledo™) | | 12 mm diameter x 215–235 mm insertion length x 13.5 PG (pipe) thread | |
| | Noise level | | < 70 dB at 1.5 m | |
| Recommended operating parameters | Operating temperature range | | Ambient to 40 ± 0.1°C (104 ± 0.2°F) | |
| | Motor speed | | 30–150 rpm | |
| | Volume range | | 250–500 L | |
| | Maximum BioProcess Container pressure | | 0.5 psi (0.03 bar) | |
| | Continuous operating time | | 21 days mixing time at nominal volume only | |

## System options

Table 3 lists available options for the 500 L S.U.B.

- **Sparger support line (Figure 3)**—keeps gas lines in an upright position for optimal gas transfer
- **Heavy-duty tubing clamps (Figure 4)**—used for each probe port not in use, eliminating process fluid holdup
- **Sterile sampling manifolds**—available in 50 and 100 mL size for off-line sample retention
- **Load cells (Figure 5)**—Mettler Toledo™ FlexMount™ load cells allow for reading of batch liquid weight; three load cells are mounted with summing box on the S.U.B. hardware unit
- **Bioreactor probe assemby (Figure 6)**—required for each sterile electrochemical probe insertion
- **Autoclave tray (Figure 7)**—aids in holding the probe assembly during the autoclave process
- **S.U.B. temperature sample port (Figure 8)**—provides off-line temperature probe calibration prior to system startup
- **Cable management tree (Figure 9)**—allows the end user to organize the S.U.B. BPC tubing lines for operator ease of use

**Table 3. 500 L S.U.B. system options.**

| Description | Cat. No. |
|---|---|
| Cable management tree | SV50992.03 |
| Load cell with summation box, without display | SV50988.03 |
| Autoclave tray | SV50177.01 |
| Bioreactor probe assembly (nonsterile for use in autoclave) with KPC connector | SH30720.01 |
| Bioreactor probe assembly (nonsterile for use in autoclave) with AseptiQuik™ connector | SH30720.02 |
| Sparger line support | SV50177B.14 |
| Heavy-duty tubing clamp (each) | SV20664.01 |
| Heavy-duty tubing clamps (10 pack) | SV20664.03 |
| Sterile sampling manifold with Luer lock (each) | SH30845.01 |
| Sterile sampling manifold with Luer lock (10 pack) | SH30845.02 |
| S.U.B. temperature/sample port | SV20750.01 |

### Additional information on autoclave tray:

- Fabricated from stainless steel
- Contains plastic carry handle for easy transport right out of the autoclave
- Positions probes on 15% incline for greater longevity
- Prevents probe bellows from collapsing during sterilization
- Accommodates two probes

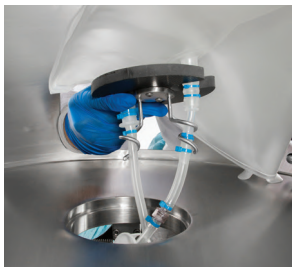


Figure 3. Sparger support line.

Figure 4. Heavy-duty tubing clamps.

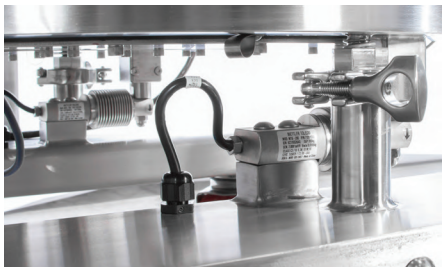


Figure 5. Load cells.

Figure 6. Bioreactor probe assembly.

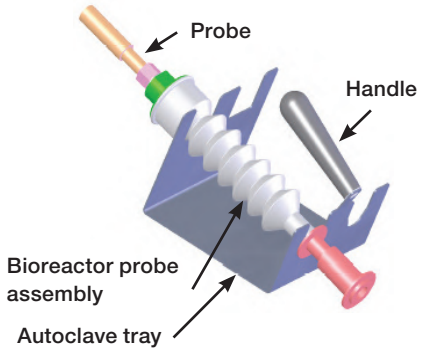



Figure 7. Autoclave tray for probe kits.

Figure 8. S.U.B. temperature sample port.

Figure 9. Cable management tree.

## Vent heaters

Vent heaters aid in reducing moisture buildup in exhaust filters from system off-gassing. Vent heaters are factory-preset at 50°C to allow condensation to return to the vessel. Recommended gassing strategies of the S.U.B. system are in the S.U.B. Validation Guide. Table 4 lists available vent heaters.

**Table 4. Vent heater required for each exhaust filter on the S.U.B. BPC.**

| Description | Voltage | Controller | Cat. No. |
|---|---|---|---|
| Meissner™ 10 in. series 46 vent filter heater | 120 VAC | Preset | SV50191.33 |
| Meissner 10 in. series 46 vent filter heater | 240 VAC | Preset | SV50191.34 |
| Meissner 10 in. series 46 vent filter heater | 120 VAC | Integrated | SV50191.47 |
| Meissner 10 in. series 46 vent filter heater | 240 VAC | Integrated | SV50191.48 |
| Pall™ Kleenpak™ KA3 series 46 vent filter heater | 120 VAC | Preset | SV50191.31 |
| Pall Kleenpak KA3 series 46 vent filter heater | 240 VAC | Preset | SV50191.32 |
| Pall Kleenpak KA3 series 46 vent filter heater | 120 VAC | Integrated | SV50191.45 |
| Pall Kleenpak KA3 series 46 vent filter heater | 240 VAC | Integrated | SV50191.46 |

## Harsh mount load cell display

Required for remote weight readout from the Mettler Toledo™ summing box, various signal output options are provided for external control monitoring (Table 5). More information can be found in the Load Cell Data Sheet.

**Table 5. Harsh mount load cell display options.**

| Description | Cat. No. |
|---|---|
| Mettler Toledo IND331 display, with analog interface (STD), 120 VAC U.S. line cord/plug | SV50177.306 |
| Mettler Toledo IND331 display, with Allen-Bradley RIO interface, 120 VAC U.S. line cord/plug | SV50177.307 |
| Mettler Toledo IND331 display, with DeviceNet interface, 120 VAC U.S. line cord/plug | SV50177.308 |
| Mettler Toledo IND331 display, with Ethernet/IP and Modbus TCP interface, 120 VAC U.S. line cord/plug | SV50177.309 |
| Mettler Toledo IND331 display, with Profibus interface, 120 VAC U.S. line cord/plug | SV50177.310 |

## Spare parts

Table 6 lists the available spare parts of the 500 L S.U.B. systems.

**Table 6. Available spare parts list.**

| Description | Cat. No. |
|---|---|
| DC motor | SV50237.22 |
| AC motor | SV50237.18 |
| Drive shaft | SV50177.36 |
| RTD 120 in. with Bulgin connector | SV50177.363 |
| Probe holders | SV50177.23 |
| Autoclave tray (stainless steel with plastic carry handle) | SV50177.01 |
| Adjustable filter bracket | SV50177.313 |

## Standard 500 L dual-sparger S.U.B. BPC systems

Table 7 shows the available dual-sparger options for the 500 L S.U.B. BPC system in either configuration: open-pipe and porous-frit spargers (Figure 10, Table 9) or drilled-hole and porous-frit spargers (Figure 11, Table 10). Standard S.U.B. BPC packaging is shown in Table 8.

**Table 7. Standard 500 L dual-sparger S.U.B. BPCs.**

| Film | Dual-sparger configuration | Cat. No. |
|---|---|---|
| CX5-14 film | Open-pipe and porous-frit spargers | SH30774.04 |
| Aegis5-14 film | Open-pipe and porous-frit spargers | SH30972.04 |
| CX5-14 film | Drilled-hole and porous-frit spargers | SH30985.04 |
| Aegis5-14 film | Drilled-hole and porous-frit spargers | SH30999.04 |

**Table 8. Standard 500 L S.U.B. BPC packaging.**

| | |
|---|---|
| **Outer packaging** | Supplied “flat-packed”<br>Two polyethylene outer layers |
| **Label** | Description<br>Product code<br>Lot number<br>Expiry date on outer packaging and shipping container |
| **Sterilization** | Irradiation (25–40 kGy) inside outer packaging |
| **Shipping container** | Durable cardboard carton |
| **Documentation** | Certificate of Analysis provided with each lot for each delivery |

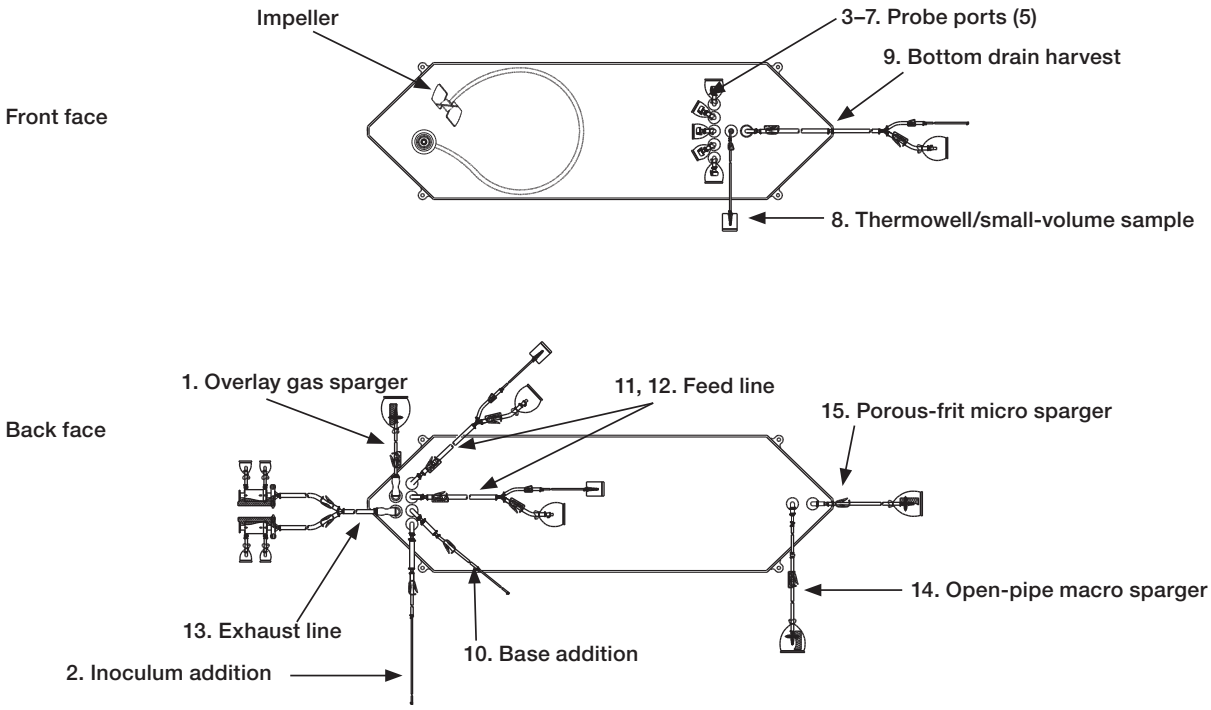



**Figure 10. Standard 500 L dual-sparger S.U.B. BPC with open-pipe and porous-frit spargers.**

**Table 9. Specifications for the standard 500 L dual-sparger S.U.B. BPC with open-pipe and porous-frit spargers.**

| Line | Description | Tubing set (inner diameter x outer diameter x length) | End treatment |
|---|---|---|---|
| 1 | Overlay gas sparger | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex™ tubing x 6 in. (15 cm) | Hydrophobic vent filter with Emflon™ II membrane |
| 2 | Inoculum addition | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 84 in. (213 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 12 in. (30 cm) | SterilEnz™ pouch with injection site assembly, 3/8 in. MPC body |
| 3–7 | Probe ports (5) | 1/2 in. (12.7 mm) tube ports | Pall™ Kleenpak™ aseptic connectors—KPCHT series (female) |
| 8 | Thermowell/small-volume sample | Thermowell adapter for 1/4 in. (6.4 mm) diameter<br>1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 24 in. (60 cm) | SterilEnz pouch with injection site assembly |
| 9 | Bottom drain harvest | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 60 in. (152 cm) reduced to 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 12 in. (30 cm) splits to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 12 in. (30 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 12 in. (30 cm) and 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 12 in. (30 cm) | Plugged<br>3/8 in. MPC insert |
| 10 | Base addition | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 84 in. (213 cm) | Plugged |
| 11, 12 | Feed lines | 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 84 in. (213 cm) splits to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 12 in. (30 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 12 in. (30 cm) and 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 12 in. (30 cm) | SterilEnz pouch with injection site assembly, 3/8 in. MPC body |
| 13 | Exhaust line | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 4 in. (10 cm) splits to 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 10 in. (25 cm) and 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 10 in. (25 cm) | (2x) Pall Kleenpak 0.2 µm exhaust vent filter |
| 14 | Open-pipe macro sparger | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 3 in. (8 cm) reduced to check valve and 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 72 in. (183 cm) | Hydrophobic vent filter with Emflon II membrane |
| 15 | Porous-frit micro sparger,<br>12 mm diameter<br>(25 µm pores) | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) reduced to check valve and 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 72 in. (183 cm) | Hydrophobic vent filter with Emflon II membrane |

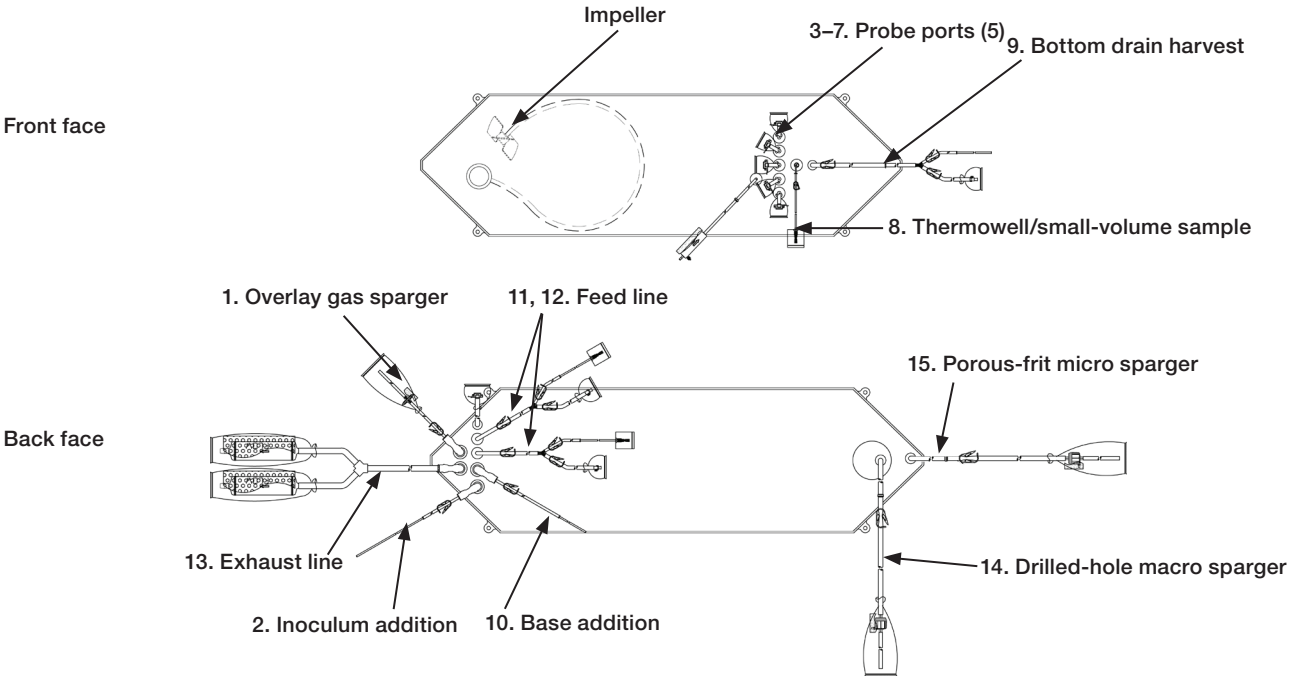



**Figure 11. Standard 500 L dual-sparger S.U.B. BPC with drilled-hole and porous-frit spargers.**

**Table 10. Specifications for the standard 500 L dual-sparger S.U.B. BPC with drilled-hole and porous-frit spargers.**

| Line | Description | Tubing set (inner diameter x outer diameter x length) | End treatment |
|---|---|---|---|
| 1 | Overlay gas sparger | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) | Meissner Steridyne™ 0.2 µm hydrophobic filter connected to 12 in. (30 cm) C-Flex tubing |
| 2 | Inoculum addition | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 84 in. (213 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 12 in. (30 cm) | Plugged |
| 3–7 | Probe ports (5) | 1/2 in. (12.7 mm) tube ports | Pall Kleenpak aseptic connectors—KPCHT series (female) |
| 8 | Thermowell/ small-volume sample | Thermowell adapter for 1/4 in. (6.4 mm) diameter 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 24 in. (60 cm) | SterilEnz pouch with injection site assembly |
| 9 | Bottom drain harvest | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 60 in. (152 cm) reduced to 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 12 in. (30 cm) splits to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 12 in. (30 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 12 in. (30 cm) and 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 12 in. (30 cm) | Plugged 3/8 in. MPC insert |
| 10 | Base addition | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 84 in. (213 cm) | Plugged |
| 11, 12 | Feed lines | 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 84 in. (213 cm) splits to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 12 in. (30 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 12 in. (30 cm) and 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 12 in. (30 cm) | SterilEnz pouch with injection site assembly, 3/8 in. MPC body |
| 13 | Exhaust line | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 12 in. (30 cm) splits to 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 6 in. (15 cm) and 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 6 in. (15 cm) | (2x) Meissner™ UltraCap™ 0.2 µm hydrophobic filters connected to 6 in. (15 cm) C-Flex tubing |
| 14 | Drilled-hole macro sparger 6.75 in. (17.1 cm) disk with 1,180 holes with a 0.018 in. (0.445 mm) diameter | 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 3 in. (8 cm) connected to check valve and 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 75 in. (190 cm) | Meissner Steridyne 0.2 µm hydrophobic filter connected to 6 in. (15 cm) C-Flex tubing |
| 15 | Porous-frit micro sparger 12 mm diameter (25 µm pores) | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) reduced to check valve and 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 78 in. (198 cm) | Meissner Steridyne 0.2 µm hydrophobic filter connected to 6 in. (15 cm) C-Flex tubing |

## Custom S.U.B. BPC options

Table 11 lists available custom 500 L S.U.B. BPC system options. Not all options are available for all ports. For additional information, please see the selection guides in the single-use products catalog.

**Table 11. Custom 500 L S.U.B. BPC options.**

| Category | Options/capability | Notes |
|---|---|---|
| **Tubing type** | Thermoplastic elastomers: C-Flex, PharMed™, PharmaPure™ tubing<br>Platinum-cured silicone<br>PVC | More information is available in the Tubing Selection Guide |
| **Tubing size** | Ranging from 1/8 to 1 in. (0.318 to 2.54 cm) ID, in customer-specified lengths | More information is available in the Tubing Selection Guide |
| **Connectors** | Luers, quick-connects, SIP connectors, tri-clamp, aseptic connectors, sterile connectors, steam-to, steam-through, sample ports, plugs | More information is available in the Connector Selection Guide. Note: Reusable probe port connections use Kleenpak™ connector only |
| **Probe ports** | Additional ports: second row of five | The reusable probe port connection uses a Kleenpak connector only |
| **Disposable sensors** | Pressure sensor: PendoTECH and Finesse Solutions<br>DO and pH: Finesse Solutions and PreSens<br>pH: Mettler Toledo | Choice of qualified sensors available |
| **Additional probe ports** | Limited engineer-to-order customization only | Qualified location on second row of probe ports only |
| **Port sizes** | Limited engineer-to-order customization only | Dependent on location in BPC and fit with hardware (e.g., 1 in. (2.54 cm) port on harvest line) |
| **Rearrangement of lines on existing ports** | Limited customization possible (e.g., moving sample/thermowell port to a probe tube port, or swapping overlay inlet line with supplement line) | Dependent on location in BPC and fit with hardware |
| **Sparger** | Dual sparger (macro open-pipe or drilled-hole and micro porous-frit) standard | Sparger locations are fixed |
| **Diptube lines** | Limited customization possible | Length cannot interfere with impeller and shaft |
| **Overlay and sparger line filters** | Filter options available from standard component library | Choice of qualified filters available |
| **Vent filters** | Standard is Pall or Meissner 0.2 µm exhaust vent filter | Filters must be compatible with available vent filter heater configurations |
| **Vent filter tubing length** | Extended filter height above the S.U.B. BPC is made to order | Must be compatible with a vent filter bracket option |
| **Filters on media and supplement inlets** | Limited engineer-to-order customization only; choice of filters used to sterilize incoming media or supplements are available | Choice of qualified filters available |

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Table 12. Recommended S.U.B. parts list for first-time operators.

| Description | Quantity | Cat. No./auxiliary part |
|---|---|---|
| S.U.B. hardware unit | 1 | Type to be configured |
| S.U.B. BPC | 3 | Type to be configured |
| Bioreactor probe assembly (nonsterile for use in autoclave) | 12 | SH30720.01 |
| Heavy-duty tubing clamp | 12 | SV20664.01 |
| Autoclave tray for autoclaving probe assemblies | 1 | SV50177.01 |
| **Auxiliary parts supporting the single-use bioreactor (supplied by end user or requested turnkey)** | | |
| Necessary for gas flow control, DO, and pH set points | 1 | Bioreactor control system |
| Autoclavable probe (13 mm x 13.5 PG thread with 195–235 mm insertion length) | * | DO probe |
| Autoclavable probe (13 mm x 13.5 PG thread with 195–235 mm insertion length) | * | pH probe |
| Tubing welder, steam-in-place system, sterilizer, or laminar flow hood | * | Sterile/aseptic connection |
| Used for fluid transfer between linesets on the containers | * | Stand-alone peristaltic pump |
| Necessary for water jacket temperature controls (not provided) | * | Temperature control unit (TCU) |

* Quantity based on needs.

## External controller options

The HyPerforma S.U.B. offers an open-architecture or turnkey system. An open-architecture system allows you to use any control system of your choice. The capital investment can be reduced by using a control system already utilized in your facility. A turnkey system is a ready-to-use, out-of-the-box system with a choice of dedicated controls from Finesse Solutions or Applikon. These systems work on PC, DeltaV, Allen-Bradley, or Siemens formats. Contact your local sales representative for more information.

Find out more at **thermofisher.com/sub**

ThermoFisher SCIENTIFIC

# HyPerforma 2:1 2,000 L Single-Use Bioreactor

## The next generation of performance

The Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) provides enhanced functionality, ease of use, and efficiency. The complete HyPerforma S.U.B. system consists of a bioreactor tank and Thermo Scientific™ HyPerforma™ S.U.B. BioProcess Container (BPC), which is available in 50, 100, 250, 500, 1,000, and 2,000 L sizes with a 2:1 turndown ratio. The redesigned HyPerforma S.U.B. maintains traditional stirred-tank bioreactor design principles including specific height-to-diameter ratios and optimized mixer location that deliver optimum cell viability, performance, and scalability from process development through production.

This data sheet provides information on the 2,000 L S.U.B. system, which includes the tank and standard S.U.B. BPC. The BPC utilizes dual-sparger configurations with a porous-frit sparger and drilled-hole or open-pipe sparger that have been rigorously tested to provide high $k_La$ values and optimal $CO_2$ stripping for improved pH control and decreased foaming.

## The S.U.B. system consists of the following components:

### S.U.B. hardware unit—available in turnkey format

- Complete mixing system with a water-jacketed vessel
- Drive shaft inserts into the S.U.B. BPC through the mixing drive motor and locks into the BPC agitator assembly
- Load cells

### S.U.B. BPC—supplied sterile and ready to use

- Agitator assembly is a single-use (polyethylene) impeller with a bearing-and-seal assembly linked to an external mixer drive
- Dual gas spargers available with either drilled-hole or open-pipe sparger and standard porous-frit sparger
- Vent filter outlet for system exhaust

- Integrally sealed ports in the S.U.B. BPC allow for addition of sensor probes and line sets
- Available in Thermo Scientific™ CX5-14 Film and Thermo Scientific™ Aegis™ 5-14 Film options

### System options—adaptable to your needs

- Optional electrical box for remote agitation control
- Optional condenser system
- Exhaust gas vent filter heaters
- Load cell displays
- Cable management tree
- Process control system
- Mobile stairs
- See Table 12 for auxiliary components for S.U.B. control management; choose an open-architecture approach or a turnkey, ready-to-use Thermo Scientific™ S.U.B. system

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## Standard S.U.B. hardware units

2,000 L standard S.U.B. hardware units are available with a water jacket only with either a DC or AC motor. The Mettler Toledo™ FlexMount™ load cells allow for batch liquid-weight reading. Three load cells are mounted with summation box on the S.U.B. hardware unit. These hardware units do not include other options listed in Tables 3–7. Base part numbers listed in Table 1 will change depending upon which options are chosen.

**Table 1. 2,000 L standard S.U.B. hardware.**

| Description | Cat. No. |
|---|---|
| Water jacketed, DC motor | SUB2000.9002 |
| Water jacketed, 240 VAC, AC motor | SUB2000.9008 |

## Design features

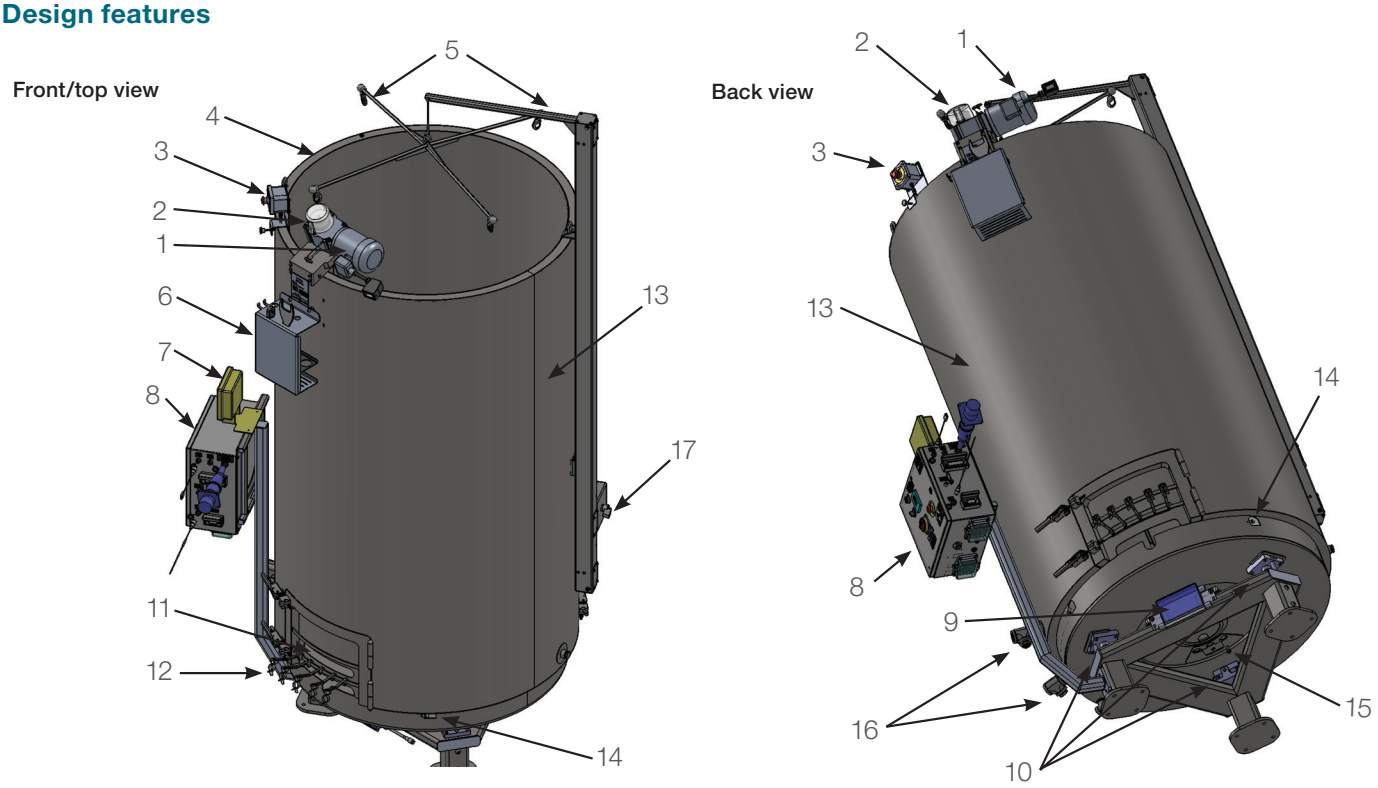


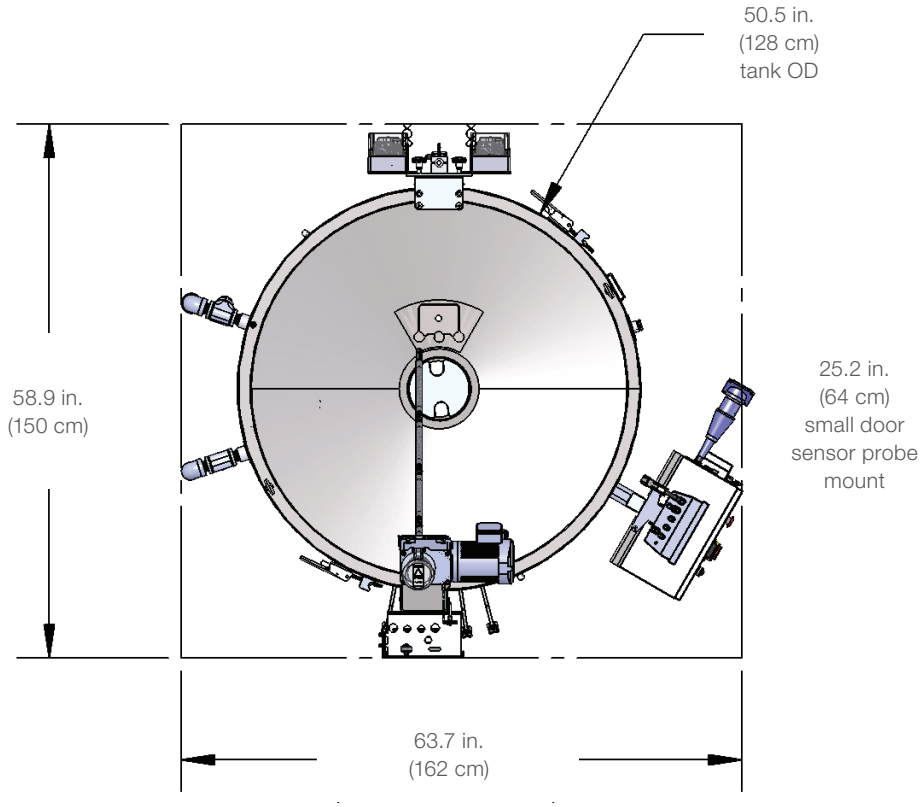

**Figure 1. 2,000 L S.U.B. hardware unit with water jacket.**

1. Mixer motor
2. Mixing assembly with shield
3. Auxiliary E-stop assembly
4. Stainless steel outer support container
5. Bag lift assembly
6. Standard tool set
7. Load cell display
8. Control panel
9. Load cell summation box
10. Load cells (3)
11. Probe access window
12. Probe clips
13. Water jacket
14. Bottom cutouts for BPC alignment
15. Sparge access plate
16. Jacket quick-connect couplings
17. Pneumatic bag lift control

**Note:** Load cells are standard on 2,000 L S.U.B. hardware units.

## Design features

Top view




Front view

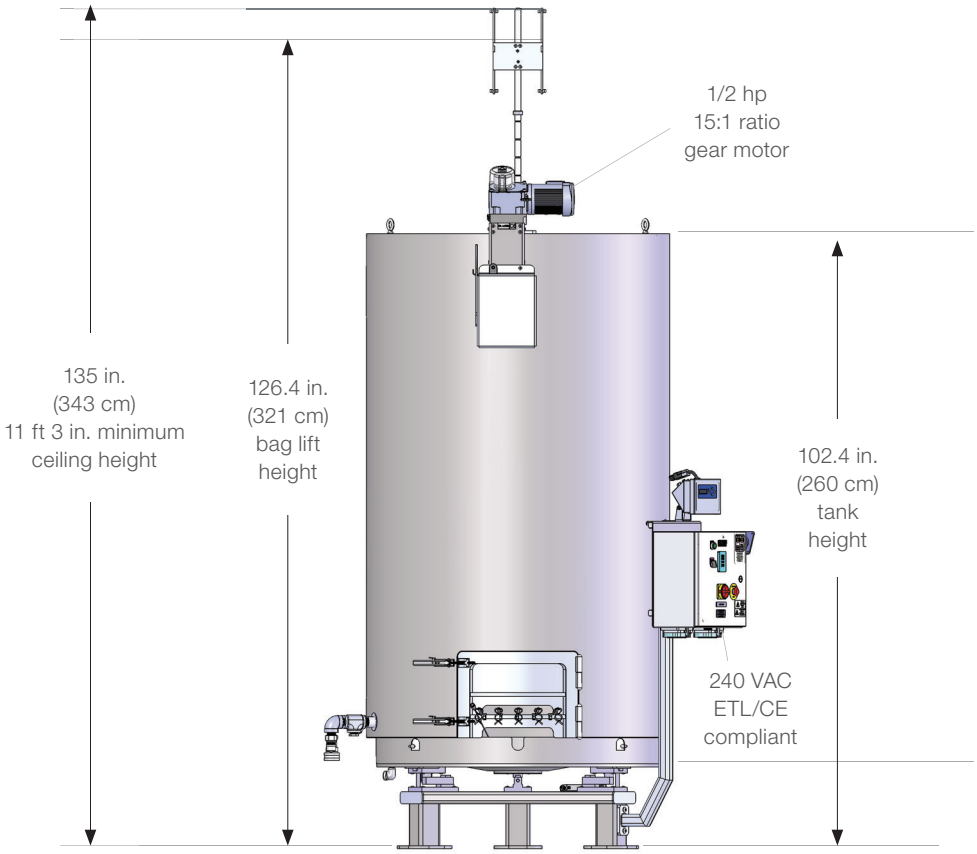



Figure 2. 2,000 L S.U.B. hardware unit dimensions.

Table 2. 2,000 L S.U.B. system specifications.

| Specifications for water-jacketed systems with AC and DC motors | | | |
|---|---|---|---|
| | | AC motor | DC motor |
| Bioreactor geometry | Rated liquid working volume | 2,000 L | |
| | Minimum liquid working volume | 1,000 L | |
| | Total bioreactor volume (liquid and gas) | 2,575 L | |
| | BPC chamber diameter | 119.4 cm (47 in.) | |
| | BPC chamber shoulder height | 229.9 cm (90.5 in.) | |
| | Liquid height at rated working volume | 178.7 cm (70.4 in.) | |
| | Fluid geometry at working volume (height:diameter ratio) | 1.5:1 | |
| | Overall bioreactor geometry (height:diameter ratio) | 1.9:1 | |
| | Tank baffles | No | |
| Impeller | Impeller (quantity x blade count) | 1 x 3 | |
| | Impeller scaling (impeller diameter/tank diameter) | 1/3 | |
| | Impeller blade pitch (angle) | 45° | |
| | Impeller diameter | 39.8 cm (15.7 in.) | |
| | Impeller calculated power number (N) | 2.1 | |
| Agitation | Maximum mixing rate | Standard: 20–75 rpm<br>Custom: up to 95 rpm above 90% working volume only | |
| | Nominal agitation rating (power/volume) | 20 W/m$^3$ | |
| | Nominal agitation | 50% working volume: 60 rpm<br>100% working volume: 75 rpm | |
| | Nominal tip speed | 154.9 cm/s (305 ft/min) | |
| | Counterclockwise mixing flow direction | Down-pumping | |
| | Agitation shaft resolved angle | 19.6° | |
| | Agitation shaft centerline offset | 6.7 cm (2.6 in.) | |
| | Overall drive shaft length (two-piece and four-piece) | 210.6 cm (82.9 in.) | |
| | Drive shaft diameter | 1.9 cm (0.8 in.) | |
| | Drive shaft poly-sheath outside diameter | 3.5 cm (1.4 in.) | |
| | Impeller clearance from tank bottom | 39.8 cm (15.7 in.) | |
| Motor | Agitation motor drive (type, voltage, phase) AC motor only | Induction, 208 VAC, 3 | – |
| | Agitation motor drive (type, voltage) DC motor only | – | Brushless, 48 VDC |
| | Motor power rating (AC motor) | 0.5 hp (372.8 W) | – |
| | Motor power rating (DC motor) | – | 0.536 hp (400 W) |
| | Motor torque rating | 27.7 N-m (245 in.-lb) | – |
| | Gear reduction | 15:1 | 20:1 |
| | Programmable VFD, remote panel interface, power fault auto restart | Standard | – |
| | Motor communication methods (for external controller) | 0–10 V, 4–20 mA, ModBus | – |

Table 2. 2,000 L S.U.B. system specifications (continued).

| Specifications for water-jacketed systems with AC and DC motors | | | | |
|---|---|---|---|---|
| | | | AC motor | DC motor |
| Temperature control | Water jacket | Jacket area: full/half-volume | 67.1/53.9 $ft^2$ | |
| | | Jacket volume | 44 L | |
| | | Jacket flow rate at 50 psi (3.4 bar) | 75 L/min | |
| | | Process connection | 1 in. male national pipe thread (NPT) nipple provided with Hansen™ quick-connect check valves | |
| | | Nominal heating/cooling load (W) | 18,000 W | |
| | | Approximate liquid heat-up time (5–37°C) | 4 hr | |
| | Misc. | RTD or thermocouple, 1/8 in. (3.18 mm) OD | RTD: Pt-100 (standard) | |
| Support container | Overall width | | 179.7 cm (70.5 in.) with E-box | 148.5 cm (58.5 in.) |
| | Overall length | | 171.4 cm (67.5 in.) | |
| | Overall height | | 321 cm (126.4 in.) | |
| | Dry skid weight (mass) | | 962.1 kg (2,121 lb) | |
| | Wet skid weight, rated working volume (mass) | | 2,962.1 kg (6,530 lb) | |
| General | Ceiling height required for 2-piece driveshaft loading | | 381 cm (150 in.) | |
| | Ceiling height required for 4-piece driveshaft loading | | 353.06 cm (139 in.) | |
| | Electrical power supply requirement (voltage, phase, current) | | 208–240 VAC, single, 10 A | Dependent on controller |
| | Tested system reliability (minimum) | | 0.9 at 90% | |
| | pH and DO probe, autoclavable type (Applisens™, Broadley James™, Mettler Toledo™) | | 12 mm diameter x 215–235 mm insertion length x 13.5 PG thread | |
| | Noise level | | < 70 dB at 1.5 m | |
| | Minimum acceleration and deceleration rate | | 60 seconds | |
| Recommended operating parameters | Operating temperature range | | Ambient to 40 ± 0.1°C (104 ± 0.2°F) | |
| | Motor speed | | Standard: 20–75 rpm<br>Custom: up to 95 rpm above 90% working volume only | |
| | Volume range | | 1,000–2,000 L | |
| | Maximum BioProcess Container pressure | | 0.5 psi (0.03 bar) | |
| | Continuous operating time | | 21 days mixing time at nominal volume only | |

## System options

Table 3 lists available S.U.B. system options for the 2,000 L size.

- **Autoclave tray (Figure 3)**—aids in holding the probe assembly during the autoclave process
- **Sparger support line (Figure 4)**—keeps gas lines in an upright position for optimal gas transfer
- **Heavy-duty tubing clamps (Figure 5)**—used for each probe port not in use, eliminating process fluid holdup
- **Sterile sampling manifolds**—available in 50 and 100 mL sizes for off-line sample retention
- **Mobile stairs (Figure 6)**—facilitates access to the bioreactor for top-mount drive shaft loading; the 2,000 L S.U.B. requires a platform for top-mount drive shaft loading (customer may provide their own solution)
- **Bioreactor probe assemblies (Figure 7)**—required for each sterile electrochemical probe insertion
- **S.U.B. temperature sample port (Figure 8)**—provides off-line temperature probe calibration prior to system startup
- **Condenser system (Figure 9)**

Table 3. 2,000 L S.U.B. system options.

| Description | Cat. No. |
|---|---|
| Complete condenser system (120 V) including cart, chill plate and mounting post with filter brackets, TCU, and pump | SV50232.01 |
| Complete condenser system (240 V) including cart, chill plate and mounting post with filter brackets, TCU, and pump | SV50232.02 |
| Autoclave tray | SV50177.01 |
| Bioreactor probe assembly (nonsterile for use in autoclave) with KPC connector | SH30720.01 |
| Bioreactor probe assembly (nonsterile for use in autoclave) with AseptiQuik™ connector | SH30720.02 |
| Sparger line support | SV50177.65 |
| Heavy-duty tubing clamp (each) | SV20664.01 |
| Heavy-duty tubing clamps (10 pack) | SV20664.03 |
| Sterile sampling manifold with Luer lock (each) | SH30845.01 |
| Sterile sampling manifold with Luer lock (10 each) | SH30845.02 |
| S.U.B. temperature/sample port | SV20750.01 |
| Mobile stairs | SV50935.01 |

### Additional information on autoclave tray:

- Fabricated from stainless steel
- Contains plastic carry handle for easy transport right out of the autoclave
- Positions probes on 15% incline for greater longevity
- Prevents probe bellows from collapsing during sterilization
- Accommodates two probes

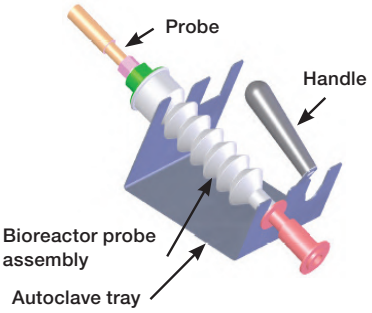



Figure 3. Autoclave tray for probe kits.

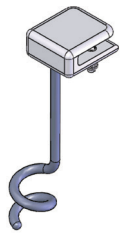


Figure 4. Sparger support line.

Figure 5. Heavy-duty tubing clamps.

Figure 6. Mobile stairs.

Figure 7. Bioreactor probe assembly.

Figure 8. S.U.B. temperature sample port.

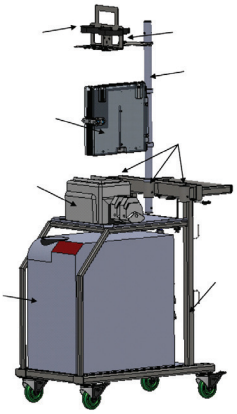


Figure 9. Condenser system.

## Vent heaters

Vent heaters aid in reducing moisture buildup in exhaust filters from system off-gassing. Vent heaters are factory-preset at 50°C to allow condensation to return to the vessel. Recommended gassing strategies of the S.U.B. system are in the S.U.B. Validation Guide. Table 4 lists available vent heaters.

**Note**: Vent heater is not required if condenser system is purchased.

**Table 4. Vent heater required for each exhaust filter on the S.U.B. BPC.**

| Description | Voltage | Controller | Cat. No. |
|---|---|---|---|
| Meissner™ 10 in. series 46 vent filter heater | 120 VAC | Preset | SV50191.33 |
| Meissner 10 in. series 46 vent filter heater | 240 VAC | Preset | SV50191.34 |
| Meissner 10 in. series 46 vent filter heater | 120 VAC | Integrated | SV50191.47 |
| Meissner 10 in. series 46 vent filter heater | 240 VAC | Integrated | SV50191.48 |
| Pall™ Kleenpak™ KA3 series 46 vent filter heater | 120 VAC | Preset | SV50191.31 |
| Pall Kleenpak KA3 series 46 vent filter heater | 240 VAC | Preset | SV50191.32 |
| Pall Kleenpak KA3 series 46 vent filter heater | 120 VAC | Integrated | SV50191.45 |
| Pall Kleenpak KA3 series 46 vent filter heater | 240 VAC | Integrated | SV50191.46 |

## Harsh mount load cell display

Required for remote weight readout from the Mettler Toledo™ summing box, various signal output options are provided for external control monitoring (Table 5). More information can be found in the Load Cell Data Sheet.

**Table 5. Harsh mount load cell display options.**

| Description | Cat. No. |
|---|---|
| Mettler Toledo IND331 display, with analog interface (STD), 120 VAC U.S. line cord/plug | SV50177.306 |
| Mettler Toledo IND331 display, with Allen-Bradley RIO interface, 120 VAC U.S. line cord/plug | SV50177.307 |
| Mettler Toledo IND331 display, with DeviceNet interface, 120 VAC U.S. line cord/plug | SV50177.308 |
| Mettler Toledo IND331 display, with Ethernet/IP and Modbus TCP interface, 120 VAC U.S. line cord/plug | SV50177.309 |
| Mettler Toledo IND331 display, with Profibus interface, 120 VAC U.S. line cord/plug | SV50177.310 |

## Spare parts

Table 6 lists the available spare parts of the 2,000 L S.U.B. systems.

**Table 6. Available spare parts list.**

| Description | Cat. No. |
|---|---|
| DC motor | SV50237.22 |
| AC motor | SV50237.19 |
| Drive shaft | SV50177.155 |
| RTD 120 in. with Bulgin connector | SV50177.363 |
| Probe holders | SV50177.23 |
| Autoclave tray (stainless steel with plastic carry handle) | SV50177.01 |

## Standard 2,000 L dual-sparger S.U.B. BPC systems

Table 7 shows the available dual-sparger options for the 2,000 L S.U.B. BPC system in either configuration: open-pipe and porous-frit spargers (Figure 10, Table 9) or drilled-hole and porous-frit spargers (Figure 11, Table 10). Standard S.U.B. BPC packaging is shown in Table 8.

**Table 7. Standard 2,000 L dual-sparger S.U.B. BPCs.**

| Film | Dual-sparger configuration | Condenser | Cat. No. |
|---|---|---|---|
| CX5-14 film | Open-pipe and porous-frit spargers | No | SH30774.07 |
| CX5-14 film | Open-pipe and porous-frit spargers | Yes | SH30774.08 |
| CX5-14 film | Drilled-hole and porous-frit spargers | Yes | SH30985.07 |
| CX5-14 film | Drilled-hole and porous-frit spargers | No | SH30985.08 |
| Aegis5-14 film | Open-pipe and porous-frit spargers | No | SH30972.07 |
| Aegis5-14 film | Open-pipe and porous-frit spargers | Yes | SH30972.08 |
| Aegis5-14 film | Drilled-hole and porous-frit spargers | Yes | SH30999.07 |
| Aegis5-14 film | Drilled-hole and porous-frit spargers | No | SH30999.08 |

**Table 8. Standard 2,000 L S.U.B. BPC packaging.**

| | |
|---|---|
| **Outer packaging** | Supplied "flat-packed"<br>Two polyethylene outer layers |
| **Label** | Description<br>Product code<br>Lot number<br>Expiry date on outer packaging and shipping container |
| **Sterilization** | Irradiation (25–40 kGy) inside outer packaging |
| **Shipping container** | Durable cardboard carton |
| **Documentation** | Certificate of Analysis provided with each lot for each delivery |

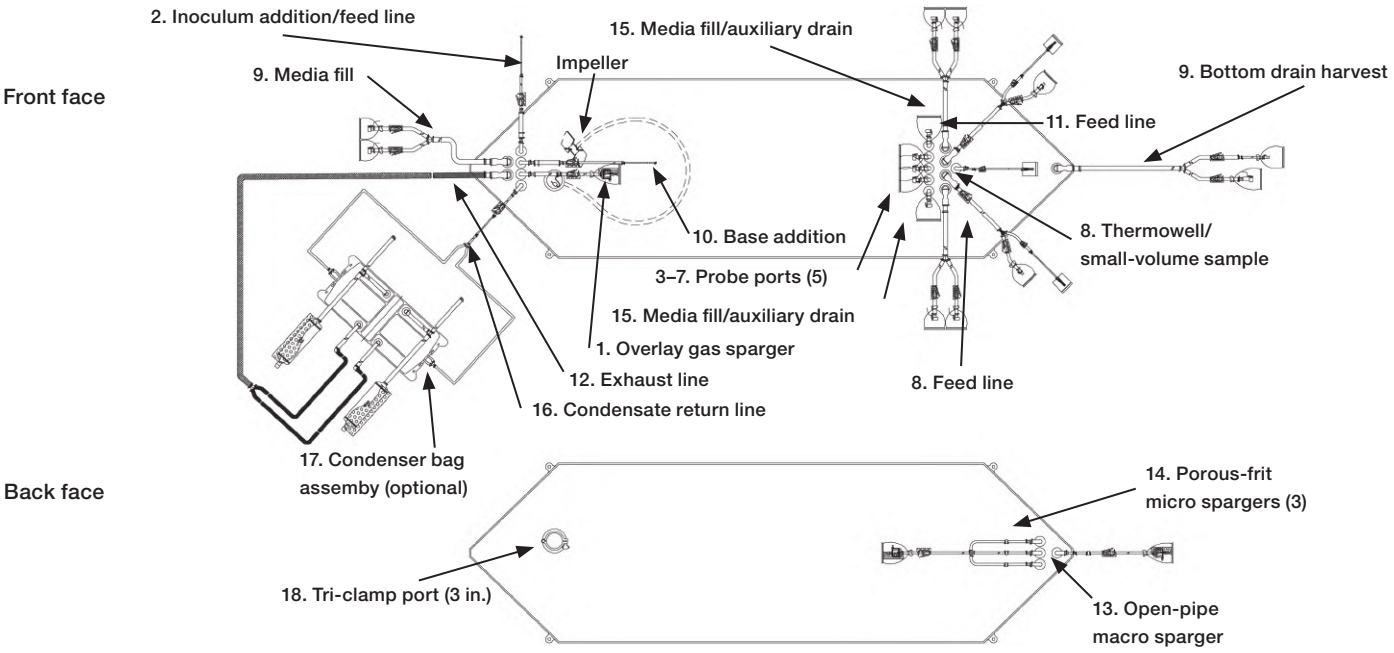



**Figure 10. Standard 2,000 L dual-sparger S.U.B. BPC with open-pipe and porous-frit spargers.** Available with or without the condenser assembly.

**Table 9. Specifications for the standard 2,000 L dual-sparger S.U.B. BPC with open-pipe and porous-frit spargers.**

| Line | Description | Tubing set (inner diameter x outer diameter x length) | End treatment |
|---|---|---|---|
| 1 | Overlay gas sparger | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex™ tubing x 4 in. (10 cm) reduced to 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 84 in. (213 cm) | Kleenpak™ Emflon™ II capsule and pressure transducer |
| 2 | Inoculum addition/ feed line | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 3 in. (8 cm) reduced to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 84 in. (213 cm) | Plugged |
| 3–7 | Probe ports (5) | 1/2 in. (12.7 mm) tube ports | Pall™ Kleenpak™ aseptic connectors (female) |
| 8 | Thermowell/ small-volume sample | Thermowell adapter for 1/4 in. (6.4 mm) diameter 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 24 in. (60 cm) | SterilEnz™ pouch with injection site assembly |
| 9 | Bottom drain harvest | 3/4 in. (19.1 mm) x 1 in. (25.4 mm) C-Flex tubing x 48 in. (122 cm) splits to 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 24 in. (61 cm) reduced to 1/4 in. (6.4 mm) x 3/8 in. (9.5 mm) C-Flex tubing x 12 in. (30 cm) and 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 24 in. (61 cm) | 1/4 in. MPC insert and Pall™ Kleenpak™ connector (male) |
| 10 | Base addition | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 3 in. (8 cm) reduced to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 84 in. (213 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 12 in. (30 cm) | Plugged |
| 11 | Feed line | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 4 in. (10 cm) splits to 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 10 in. (25 cm) and 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 10 in. (25 cm) | SterilEnz pouch with injection site assembly and 3/8 in. MPC body |
| 12 | Exhaust line | Condenser bag assembly (optional) | – |
| 13 | Open-pipe macro sparger | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 72 in. (183 cm) reduced to check valve and 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 72 in. (183 cm) | Kleenpak Emflon II capsule |
| 14 | Porous-frit micro spargers (3), 12 mm diameter (25 µm pores) | (2x) 12 mm PDVF porous sparge inserts connected to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) converge to one 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 72 in. (183 cm) | Kleenpak Emflon II capsule |
| 15 | Media fill/ auxiliary drain | 3/4 in. (19.1 mm) x 1 in. (25.4 mm) C-Flex tubing x 84 in. (213 cm) splits to 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 24 in. (61 cm) and 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 24 in. (61 cm) | Pall Kleenpak connectors (female) |
| 16 | Condensate return line | Condenser bag assembly (optional) | – |
| 17 | Condenser bag assembly | Condenser bag assembly (optional) | – |
| 18 | Tri-clamp port (3 in.) | NA | Gasket, end cap, and clamp |

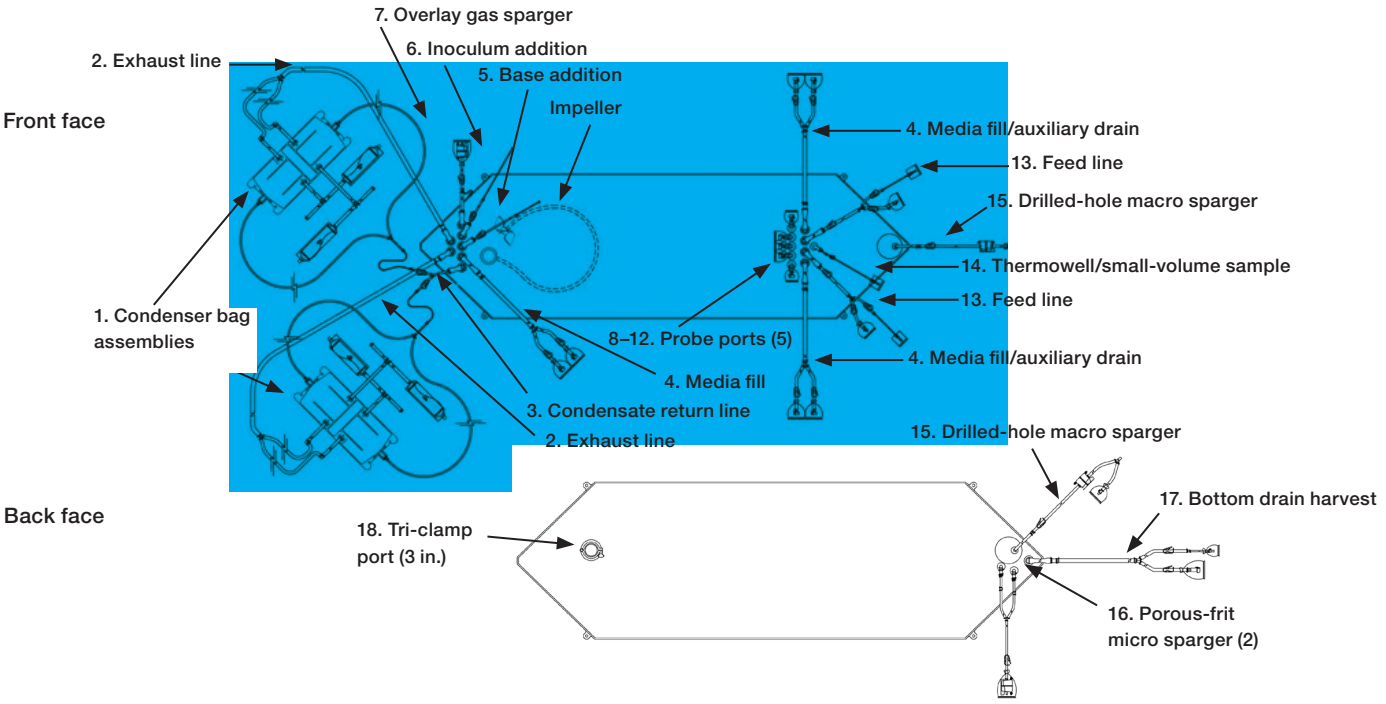



**Figure 11. Standard 2,000 L dual-sparger S.U.B. BPC with drilled-hole and porous-frit spargers.** Available with or without condenser assemblies.

**Table 10. Specifications for the standard 2,000 L dual-sparger S.U.B. BPC with drilled-hole and porous-frit spargers.**

| Line | Description | Tubing set (inner diameter x outer diameter x length) | End treatment |
|---|---|---|---|
| 1 | Condenser bag assemblies (2) | Condenser bag assembly (optional) | – |
| 2 | Exhaust lines (2) | Condenser bag assembly (optional) | – |
| 3 | Condensate return line | Condenser bag assembly (optional) | – |
| 4 | Media fill/auxiliary drain lines | (3x) 3/4 in. (19.1 mm) x 1 in. (25.4 mm) C-Flex tubing x 84 in. (213 cm) splits to 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 24 in. (61 cm) and 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 24 in. (61 cm) | Pall Kleenpak aseptic connectors (female) |
| 5 | Base addition | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 3 in. (8 cm) reduced to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 84 in. (213 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 12 in. (30 cm) | Plugged |
| 6 | Inoculum addition/ feed line | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 3 in. (8 cm) reduced to 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) reduced to 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 84 in. (213 cm) | Plugged |
| 7 | Overlay gas sparger | 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 4 in. (10 cm) reduced to 3/8 in. (9.5 mm) x 5/8 in. (15.9 mm) C-Flex tubing x 84 in. (213 cm) | Kleenpak Emflon II capsule and pressure transducer |
| 8–12 | Probe ports (5) | 1/2 in. (12.7 mm) tube ports | Pall Kleenpak aseptic connectors (female) |
| 13 | Feed lines | (2x) 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 4 in. (10 cm) splits to 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 10 in. (25 cm) and 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 10 in. (25 cm) | SterilEnz pouch with injection site assembly and 3/8 in. MPC body |
| 14 | Thermowell/ small-volume sample | Thermowell adapter for 1/4 in. (6.4 mm) diameter 1/8 in. (3.2 mm) x 1/4 in. (6.4 mm) C-Flex tubing x 24 in. (61 cm) | SterilEnz pouch with injection site assembly |
| 16 | Porous-frit micro sparger, 12 mm diameter (25 µm pores) | 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 6 in. (15 cm) reduced to check valve and 1/4 in. (6.4 mm) x 7/16 in. (11.1 mm) C-Flex tubing x 72 in. (183 cm) | (2x) Meissner™ Steridyne™ 0.2 µm hydrophobic filter |
| 17 | Bottom drain harvest | 3/4 in. (19.1 mm) x 1 in. (25.4 mm) C-Flex tubing x 48 in. (122 cm) splits to 1/2 in. (12.7 mm) x 34 in. (19.1 mm) C-Flex tubing x 24 in. (61 cm) reduced to 1/4 in. (6.4 mm) x 3/8 in. (9.5 mm) C-Flex tubing x 12 in. (30 cm) and 1/2 in. (12.7 mm) x 3/4 in. (19.1 mm) C-Flex tubing x 24 in. (61 cm) | 1/4 in. MPC insert and Pall Kleenpak connector (male) |
| 18 | Tri-clamp port (3 in.) | NA | Gasket, end cap, and clamp |

## Custom S.U.B. BPC options

Table 11 lists available custom 2,000 L S.U.B. BPC system options. Not all options are available for all ports. For additional information, please see the selection guides in the single-use products catalog.

**Table 11. Custom 2,000 L S.U.B. BPC options.**

| Category | Options/capability | Notes |
|---|---|---|
| **Tubing type** | Thermoplastic elastomers: C-Flex, PharMed™, PharmaPure™ tubing<br>Platinum-cured silicone<br>PVC | More information is available in the Tubing Selection Guide |
| **Tubing size** | Ranging from 1/8 to 1 in. (0.318 to 2.54 cm) ID, in customer-specified lengths | More information is available in the Tubing Selection Guide |
| **Connectors** | Luers, quick-connects, SIP connectors, tri-clamp, aseptic connectors, sterile connectors, steam-to, steam-through, sample ports, plugs | More information is available in the Connector Selection Guide. Note: Reusable probe port connections use Kleenpak™ connector only |
| **Probe ports** | Additional ports: second row of five | The reusable probe port connection uses a Kleenpak connector only |
| **Disposable sensors** | Pressure sensor: PendoTECH and Finesse Solutions<br>DO and pH: Finesse Solutions and PreSens<br>pH: Mettler Toledo | Choice of qualified sensors available; PendoTECH pressure sensors come standard |
| **Additional probe ports** | Limited engineer-to-order customization only | To be designed |
| **Port sizes** | Limited engineer-to-order customization only | Dependent on location in BPC and fit with hardware (e.g., 1 in. (2.54 cm) port on harvest line) |
| **Rearrangement of lines on existing ports** | Limited customization possible (e.g., moving sample/thermowell port to a probe tube port, or swapping overlay inlet line with supplement line) | Dependent on location in BPC and fit with hardware |
| **Sparger** | Dual sparger (macro open-pipe or drilled-hole and micro porous-frit) standard | Sparger locations are fixed |
| **Diptube lines** | Limited customization possible | Length cannot interfere with impeller and shaft |
| **Overlay and sparger line filters** | Filter options available from standard component library | Choice of qualified filters available |
| **Vent filters** | Standard is Pall or Meissner 0.2 µm exhaust vent filter | Filters must be compatible with available vent filter heater configurations |
| **Vent filter tubing length** | Extended filter height above the S.U.B. BPC is made to order | Must be compatible with a vent filter bracket option |
| **Filters on media and supplement inlets** | Limited engineer-to-order customization only; choice of filters used to sterilize incoming media or supplements are available | Choice of qualified filters available |

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Table 12. Recommended S.U.B. parts list for first-time operators.

| Description | Quantity | Cat. No./auxiliary part |
|---|---|---|
| S.U.B. hardware unit | 1 | Type to be configured |
| S.U.B. BPC | 3 | Type to be configured |
| Bioreactor probe assembly (nonsterile for use in autoclave) | 12 | SH30720.01 |
| Heavy-duty tubing clamp | 12 | SV20664.01 |
| Autoclave tray for autoclaving probe assemblies | 1 | SV50177.01 |
| **Auxiliary parts supporting the single-use bioreactor (supplied by end user or requested turnkey)** | | |
| Necessary for gas flow control, DO, and pH set points | 1 | Bioreactor control system |
| Autoclavable probe (13 mm x 13.5 PG thread with 195–235 mm insertion length) | * | DO probe |
| Autoclavable probe (13 mm x 13.5 PG thread with 195–235 mm insertion length) | * | pH probe |
| Tubing welder, steam-in-place system, sterilizer, or laminar flow hood | * | Sterile/aseptic connection |
| Used for fluid transfer between linesets on the containers | * | Stand-alone peristaltic pump |
| Necessary for water jacket temperature controls (not provided) | * | Temperature control unit (TCU) |

* Quantity based on needs.

## External controller options

The HyPerforma S.U.B. offers an open-architecture or turnkey system. An open-architecture system allows you to use any control system of your choice. The capital investment can be reduced by using a control system already utilized in your facility. A turnkey system is a ready-to-use, out-of-the-box system with a choice of dedicated controls from Finesse Solutions or Applikon. These systems work on PC, DeltaV, Allen-Bradley, or Siemens formats. Contact your local sales representative for more information.

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Validation Guide

# Thermo Scientific HyPerforma Single-Use Bioreactor Systems Validation Guide

**Revision A**

DOC0016 • December 2015

Thermo SCIENTIFIC

# 2.6 Oxygen Transfer

## Introduction

The S.U.B. BPC is designed to provide an acceptable range of $k_La$ values to support rapid growth for an array of common cell platforms using the operating parameters shown in Table 2.7 for BPCs equipped with open pipe and porous frit spargers, and Table 2.8 for BPCs equipped with drilled hole and porous frit spargers.

| S.U.B. BPCs Range of Operating Parameters with Open Pipe and Frit Spargers | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50L | | | 100L | | | 250L | | | 500L | | | 1,000L | | | 2,000L | | |
| Temperature (°C) | 2.0 - 40.0 ± 0.1 | | | | | | | | | | | | | | | | | |
| Operating Volume (L) | 25 to 50 | | | 50 to 100 | | | 125 to 250 | | | 250 to 500 | | | 500 to 1,000 | | | 1,000 to 2,000 | | |
| Agitation Rate (rpm) | 30 to 200 | | | 30 to 200 | | | 30 to 150 | | | 30 to 150 | | | 20 to 110 | | | 20 to 75 | | |
| Recommended Max. Gas Flow Rates | Open Pipe | Porous Frit | Overlay | Open Pipe | Porous Frit | Overlay | Open Pipe | Porous Frit | Overlay | Open Pipe | Porous Frit | Overlay | Open Pipe | Porous Frit | Overlay | Open Pipe | Porous Frit (3) | Overlay |
| Air (slpm) | 1 | 0.5 | 5 | 2 | 1 | 10 | 5 | 2.5 | 10 | 10 | 5 | 15 | 10 | 8 | 15 | 12 | 16 | 15 |
| $O_2$ (slpm) | - | 0.25 | - | - | 0.5 | - | - | 1.25 | - | - | 2.5 | - | - | 4 | - | - | 8 | - |
| $CO_2$ (slpm) | - | 0.1 | - | - | 0.2 | - | - | 0.5 | - | - | 1 | - | - | 1 | - | - | 1 | - |
| $N_2$ (slpm) | - | 0.25 | - | - | 0.5 | - | - | 1.25 | - | - | 2.5 | - | - | 2.5 | - | - | 2.5 | - |
| Total (slpm) | 1 | 0.85 | 5 | 2 | 1.7 | 10 | 5 | 4.25 | 10 | 10 | 8.5 | 15 | 10 | 13 | 15 | 12 | 25 | 15 |
| Exhaust Load (slpm) | 20 | | | 20 | | | 20 | | | 40 | | | 40 | | | 90 | | |

**Table 2.8** Operating parameters using open pipe and porous frit spargers

| S.U.B. BPCs Range of Operating Parameters with Drilled Hole and Frit Spargers | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50L | | | 100L | | | 250L | | | 500L | | | 1,000L | | | 2,000L | | |
| Temperature (°C) | 2.0 - 40.0 ± 0.1 | | | | | | | | | | | | | | | | | |
| Operating Volume (L) | 25 to 50 | | | 50 to 100 | | | 125 to 250 | | | 250 to 500 | | | 500 to 1,000 | | | 1,000 to 2,000 | | |
| Agitation Rate (rpm) | 30 to 200 | | | 30 to 200 | | | 30 to 150 | | | 30 to 150 | | | 20 to 110 | | | 20 to 75[1] | | |
| Recommended Max. Gas Flow Rates | Drilled Hole | Porous Frit | Overlay | Drilled Hole | Porous Frit | Overlay | Drilled Hole | Porous Frit | Overlay | Drilled Hole | Porous Frit | Overlay | Drilled Hole | Porous Frit | Overlay | Drilled Hole | Porous Frit | Overlay |
| Air (slpm) | 2.5 | 1 | 5 | 5 | 2 | 10 | 12 | 4 | 14 | 25 | 6 | 35 | 100 | 8 | 60 | 200 | 16 | 129[2] |
| $O_2$ (slpm) | - | 1 | - | - | 2 | - | - | 4 | - | - | 6 | - | - | 8 | - | - | 16 | - |
| $CO_2$ (slpm) | - | 0.25 | - | - | 0.5 | - | - | 1 | - | - | 1.5 | - | - | 2 | - | - | 4 | - |
| $N_2$ (slpm) | - | 1 | - | - | 2 | - | - | 4 | - | - | 6 | - | - | 8 | - | - | 16 | - |
| Total (slpm) | 2.5 | 1.25 | 5 | 5 | 2.5 | 10 | 12 | 5 | 14 | 25 | 7.5 | 35 | 100 | 10 | 60 | 200 | 20 | 129 |
| Exhaust Load (slpm) | 20 | | | 20 | | | 90 | | | 90 | | | 180 | | | 270 | | |

**Table 2.9** Operating parameters using drilled hole and porous frit spagers

## Study Method

Experiments were designed to estimate and model mass transfer of gasses in S.U.B. systems. For more information about methods and procedures, see the test methods detail in section 6.4 in the appendix of this manual.

## Results Overview

Experiments were performed using 50, 250 and 2,000L vessels to measure the mass transfer of oxygen and $CO_2$ stripping and the results for 100, 500 and 1,000L vessel sizes have been interpolated theoretically from those results.

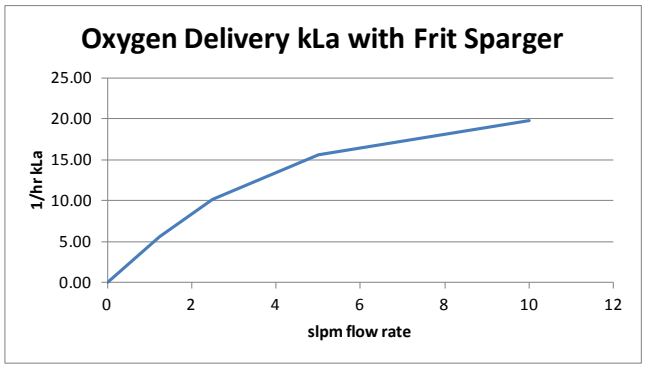
The results in this section show the mass transfer of oxygen and $CO_2$ stripping, and are presented as $k_La$ for various sparge flow rates for each vessel size. Two dimensional plots are used to show individual sparger results for oxygen delivery and $CO_2$ stripping, separately. Three dimensional plots are used to show the combined micro/macro (porous frit/open pipe or porous frit/drilled hole) sparger oxygen delivery behavior in terms of $k_La$ response at different combined flow rates for each vessel size.

Results, unless otherwise specified, are at an agitation power input per volume (PIV) of 0.15 HP/1,000gal (29.6 W/m3).

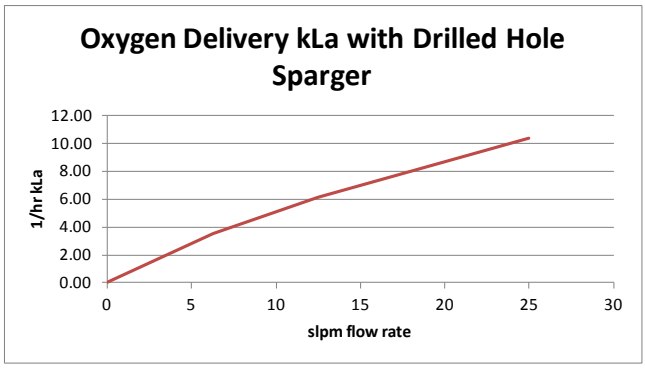
Results for vessels using porous frit and open pipe spargers are presented first. Results for vessels using porous frit and Drilled Hole Spargers are presented in separate, subsequent sections.

## 500L Results for Porous Frit and Drilled Hole Spargers

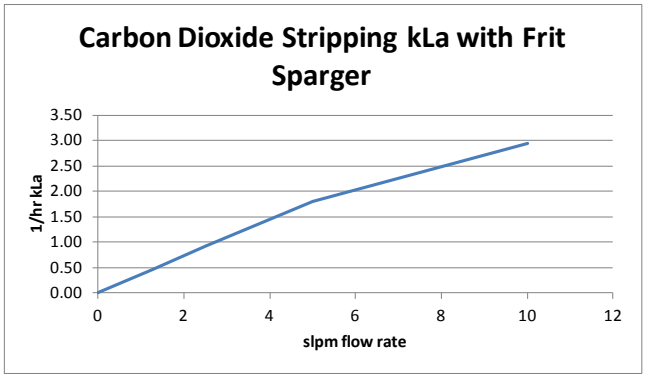
The data shown below for 500L vessels is estimated, and has been interpolated from experimentally-derived 250 and 2,000L data and biased by pore diameter of the drilled hole spargers.



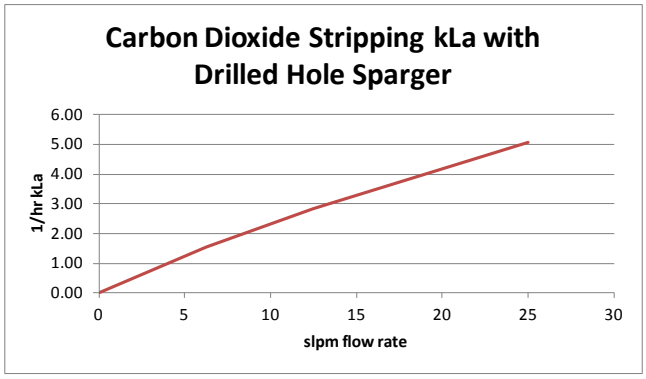

**Graph 2.47** Interpolated Results for 500L S.U.B. with porous frit sparger



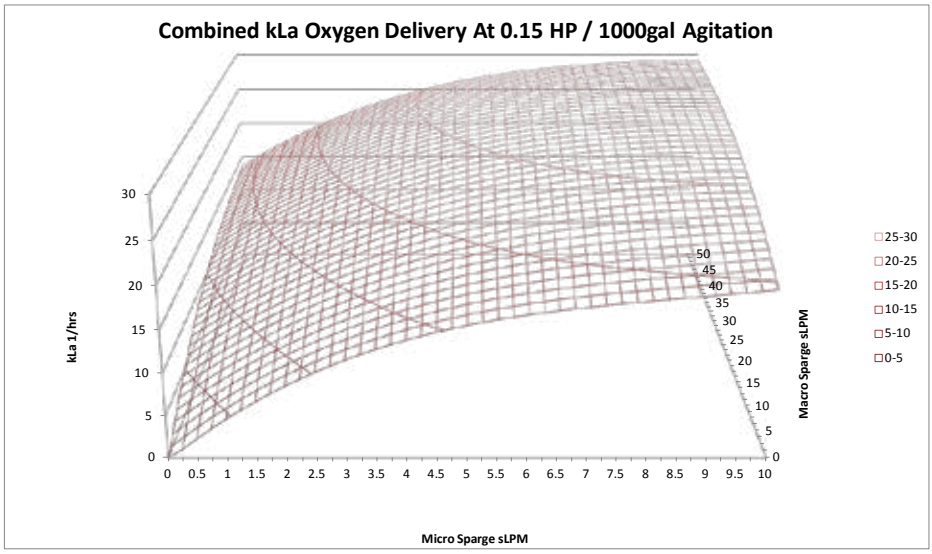

**Graph 2.48** Interpolated Results for 500L S.U.B. with drilled hole sparger




**Graph 2.49** Interpolated Results for 500L S.U.B. with porous frit sparger



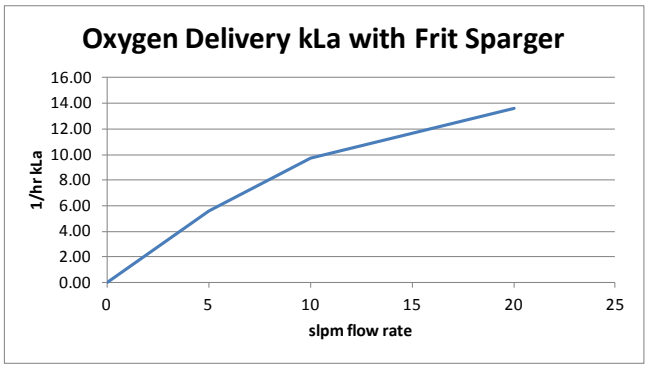

**Graph 2.50** Interpolated Results for 500L S.U.B. with drilled hole sparger



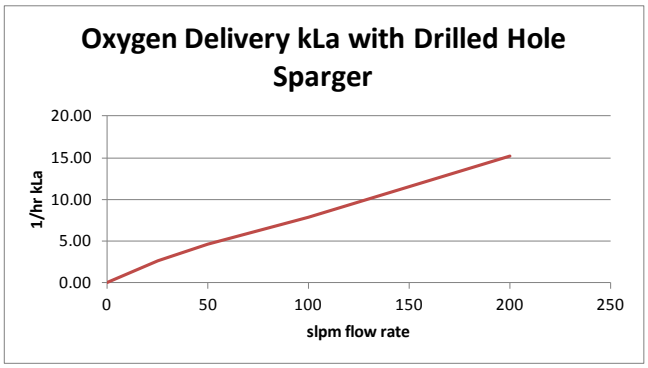

**Graph 2.51** Interpolated Results for 500L S.U.B. with both porous frit and drilled hole spargers

## 2,000L Results for Frit and Drilled Hole Spargers

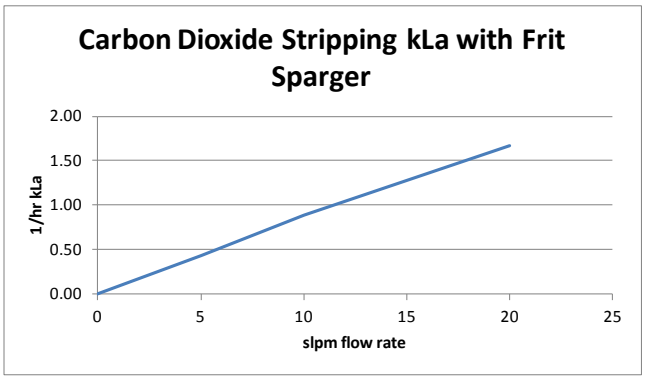
The results of experiments with 2,000L vessels using porous frit and drilled hole spargers are shown below.



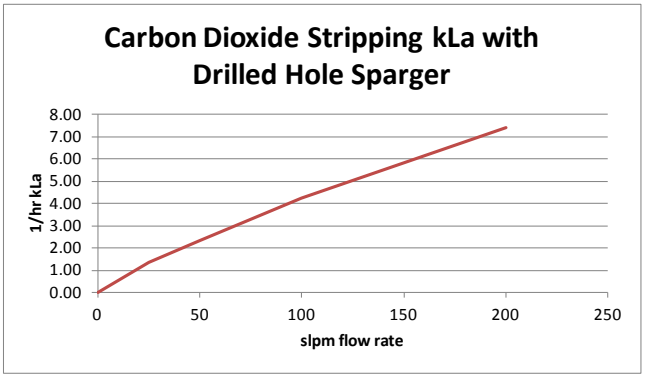

**Graph 2.57** Results for 2,000L S.U.B. with porous frit sparger



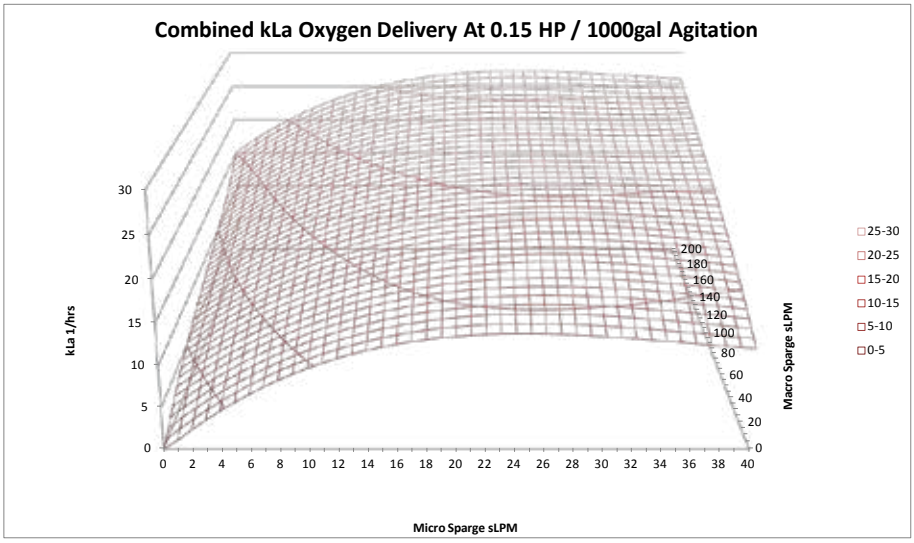

**Graph 2.58** Results for 2,000L S.U.B. with drilled hole sparger




**Graph 2.59** Results for 2,000L S.U.B. with porous frit sparger




**Graph 2.60** Results for 2,000L S.U.B. with drilled hole sparger




**Graph 2.61** Results for 2,000L S.U.B. with both porous frit and drilled hole spargers

## 2.7 Mixing Studies

The recommended range of mixing rates for the range of S.U.B. systems is as follows:

| | 50L | 100L | 250L | 500L | 1,000L | 2,000L |
|---|---|---|---|---|---|---|
| Operating Volume (L) | 25-50 | 50-100 | 125-250 | 250-500 | 500-1,000 | 1,000-2,000 |
| Agitation Rate (rpm) | 30-200 | 30-200 | 30-150 | 30-150 | 20-110 | 20-75 |

**Table 2.10** Recommended range of mixing rates

### Study Method (50 to 1,000L)

The mixing efficiency was estimated for the range of agitation rates by measuring the conductivity of the liquid contents of the S.U.B. BPC at different locations within the system after the addition of sodium chloride solution. Conductivity was measured with three conductivity probes positioned at the top, middle and bottom.

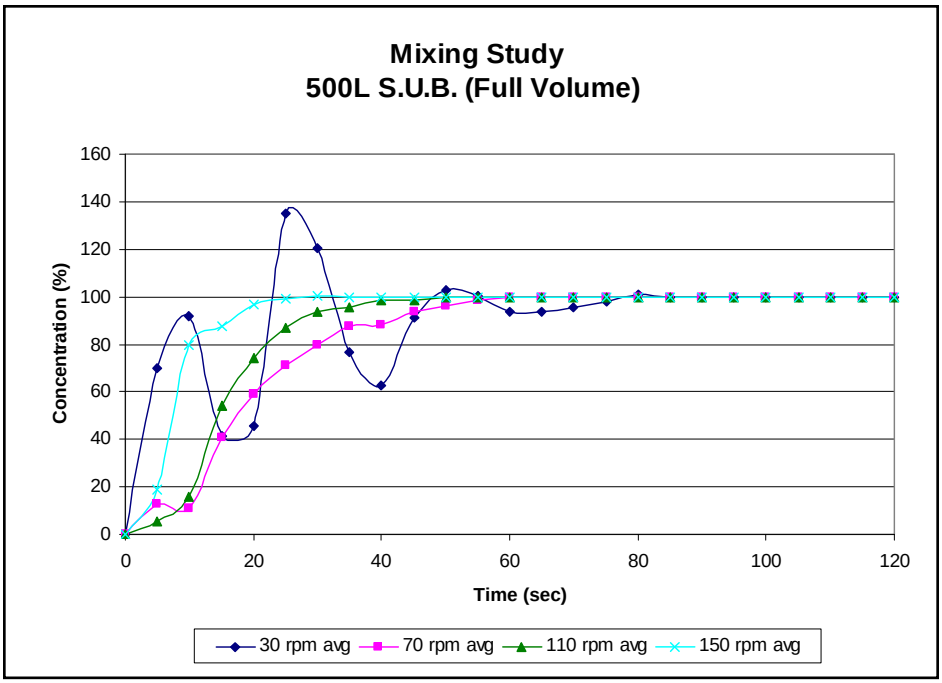
The time to achieve uniform distribution of sodium chloride throughout the BPC was designated as the mixing time. Since multiple sensors were used the average time was determined when concentration readings of all the sample locations achieved a minimum of 95% of the final concentration. The study was conducted at maximum and minimum operating volumes for 50, 100, 250, 500 and 1,000L S.U.B. systems.

| 50L S.U.B. | | | | 100L S.U.B. | | | |
|---|---|---|---|---|---|---|---|
| Half Volume | | Full Volume | | Half Volume | | Full Volume | |
| Agitation Speed (rpm) | Mixing Time (sec) | Agitation Speed (rpm) | Mixing Time (sec) | Agitation Speed (rpm) | Mixing Time (sec) | Agitation Speed (rpm) | Mixing Time (sec) |
| 50 | 80 | 50 | 150 | 50 | 55-60 | 50 | 80-85 |
| 100 | 20 | 100 | 50 | 100 | 30-35 | 100 | 40-45 |
| 150 | 15 | 150 | 40 | 150 | 20-25 | 150 | 35-40 |
| | | 200 | 10 | | | 200 | 30-35 |

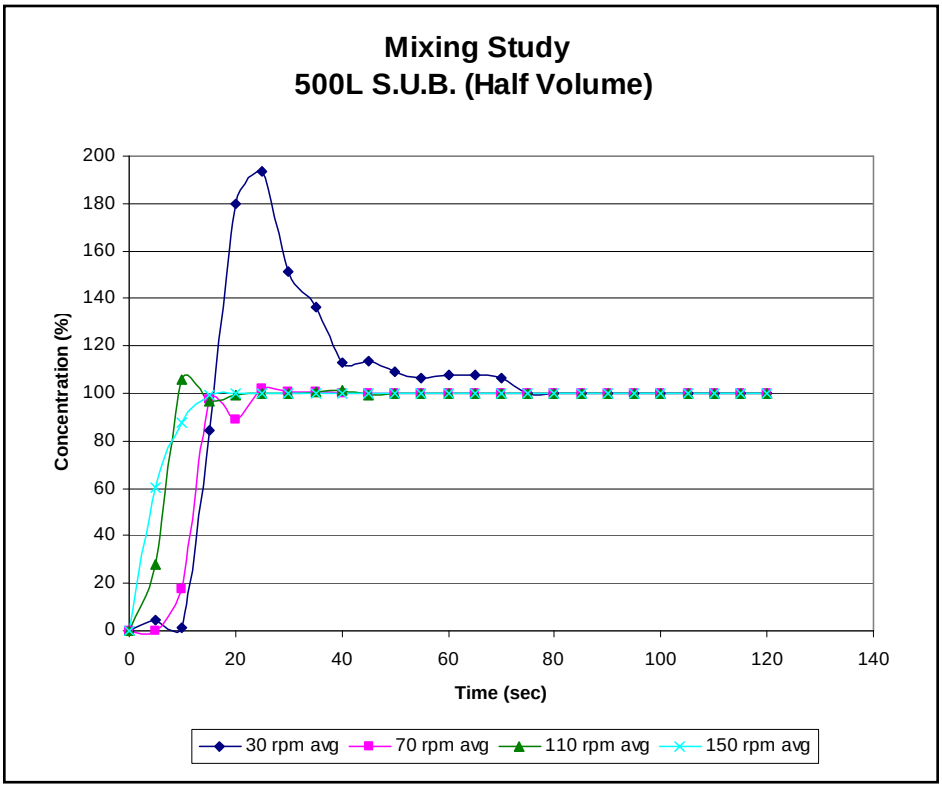
| 250L S.U.B. | | | | 500L S.U.B. | | | |
|---|---|---|---|---|---|---|---|
| Half Volume | | Full Volume | | Half Volume | | Full Volume | |
| Agitation Speed (rpm) | Mixing Time (sec) | Agitation Speed (rpm) | Mixing Time (sec) | Agitation Speed (rpm) | Mixing Time (sec) | Agitation Speed (rpm) | Mixing Time (sec) |
| 40 | 50 | 60 | 60 | 30 | 75 | 30 | 80 |
| 60 | 30 | 100 | 40 | 70 | 25 | 70 | 60 |
| 80 | 20 | 120 | 30 | 110 | 20 | 110 | 50 |
| | | 140 | 20 | 150 | 15 | 150 | 25 |

| 1,000L S.U.B. | | | |
|---|---|---|---|
| Half Volume | | Full Volume | |
| Agitation Speed (rpm) | Mixing Time (sec) | Agitation Speed (rpm) | Mixing Time (sec) |
| 30 | 68-89 | 60 | 37-47 |
| 45 | 37-40 | 70 | 30-39 |
| 60 | 35-39 | 80 | 30-43 |
| | | 90 | 26-34 |
| | | 100 | 20-29 |
| | | 110 | 19-28 |

**Table 2.11** Summary of mixing study results for the 50 to 1,000L S.U.B. systems




**Graph 2.68** Mixing study 500L S.U.B. – full volume




**Graph 2.69** Mixing study 500L S.U.B. – full volume

# Mixing Study (2,000L)

## Theory

Mixing performance was evaluated using an electrolyte solution and conductivity sensors. These sensors offer a very fast response time and both stable and repeatable readings. Mixing time is defined as the time elapsed between when stock solution is added and the average sample location reading exceeds 95% of final concentration. These mixing tests represent best case time estimates as the salt is added as a pre-mix solution.

## Procedure

Each bag was filled with DI water to the test volume, heated to 40ºC, and a salt solution was prepared. For the tests a 1 liter volume of solution (300 grams per liter dissolved Sodium Chloride) was introduced at the top of the BPC. Each test consisted of verifying the correct agitation speed and starting values of conductivity, adding the appropriate amount of salt solution, and then recording the readings on the probes utilizing the Kaye Validator™ thermal validation system until the conductivity leveled off. After the data were collected and entered, the percentages compared to the final reading were calculated for each sample taken. The percentage values from the probes were then averaged to approximate the mixing time. All three probes were used for both full and half volume calculations.

Three conductivity sensors from the same model and manufacture were attached to a rod installed into the BPC from the top. These sensors were positioned next to the top mounted mixer drive motor. It is anticipated that the location on this side of the tank represents worst case mixing because they are located the greatest distance from the high shear region of the impeller. The sensor positions represent three column height locations of low, middle, and high. The low and high positions were each located approximately 30.5cm (12.0”) from the respective ends of the fluid column. The mid probe was located at the 1,000L mark (half volumes). In an effort to obtain a representative reading the sensor tips protruded into the tank no less than 2.54cm (1.0”) from the inside of the tank wall. For half volume mix tests, the low and high probes were located approximately 15.2cm (6.0”) from the respective ends of the fluid column and the mid probe was located at the 500L mark.

| Mixing Test #* | Tank Volume | Impeller Location/Shaft Length | Power/Vol (Hp/1,000 gal) | RPM |
|---|---|---|---|---|
| M1 | Nominal, 2,000L | 1 diameter from bottom / 82.9” | 0.05 | 60 |
| M2 | Nominal, 2,000L | 1 diameter from bottom / 82.9” | 0.1 | 75 |
| M3 | Nominal, 2,000L | 1 diameter from bottom / 82.9” | 0.2 | 95 |
| M10 | ½ Volume, 1,000L | 1 diameter from bottom / 82.9” | 0.05 | 47 |
| M11 | ½ Volume, 1,000L | 1 diameter from bottom / 82.9” | 0.1 | 60 |
| M12 | ½ Volume, 1,000L | 1 diameter from bottom / 82.9” | 0.2 | 75 |

**Table 2.12** Mixing study test matrix 2,000 L S.U.B.

## Results

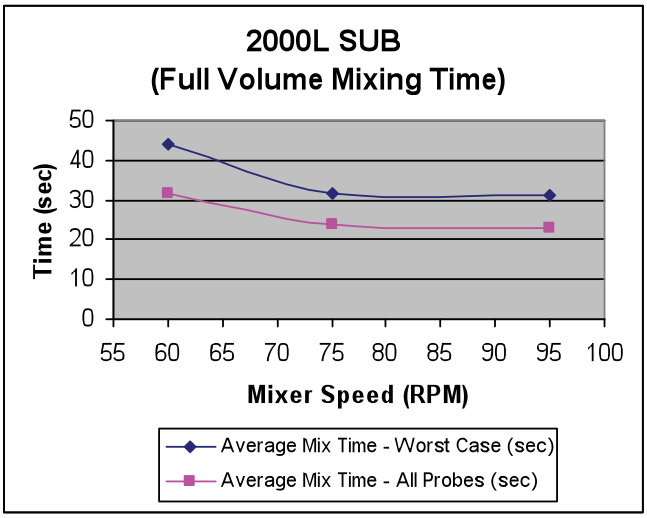
All tests conducted were done in duplicate. The first column in results Table 2.13, below, was calculated by determining which probe (top, mid, or bottom) resulted in the slowest mix time for each respective test and averaging those together. The second column was calculated by averaging the all of the probes.

Mixing time performance at full volume is near equivalent at 75 and 95rpm.

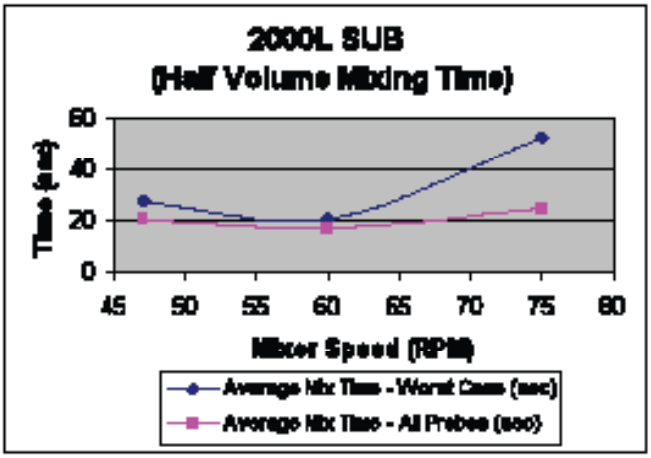
Mixing at high impeller speed (>60rpm) at half volume is not recommended and will result in less than desirable performance and accelerated shaft wear (excessive power input, poor power dissipation result in lack of turn-over in a non-baffled tank).

| 2,000L S.U.B. (Full Volume Mixing Time) | | |
|---|---|---|
| **RPM** | **Average Mix Time - Worst Case (sec)** | **Average Mix Time - All Probes (sec)** |
| 60 | 44 | 31.5 |
| 75 | 31.5 | 24 |
| 95 | 31 | 22.8 |
| **2,000L S.U.B. (Half Volume Mixing Time)** | | |
| **RPM** | **Average Mix Time - Worst Case (sec)** | **Average Mix Time - All Probes (sec)** |
| 47 | 28 | 20.8 |
| 60 | 21 | 16.8 |
| 75 | 53 | 24.8 |

**Table 2.13** Mixing study results 2,000L S.U.B.




**Graph 2.72** Mixing study 2,000L S.U.B. - full volume

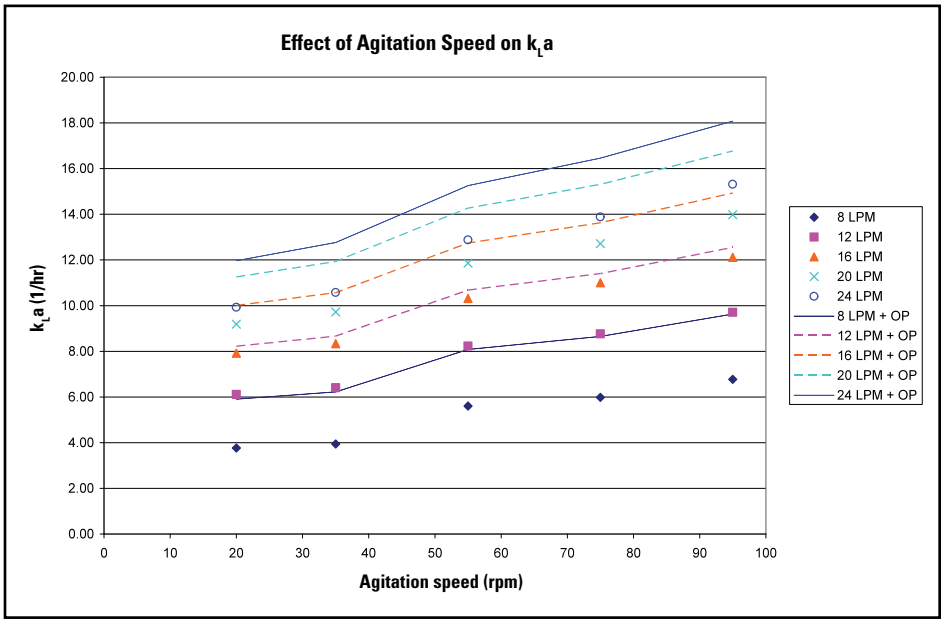



**Graph 2.73** Mixing study 2,000L S.U.B. - half volume

| 2,000L S.U.B. | | | | | | | |
|---|---|---|---|---|---|---|---|
| Porous Frit Sparger Only | | | | Porous Frit Sparger Plus 0.01 VVM (10 LPM) Through Pipe | | | |
| RPM | Frit Flow (lpm) | Open Pipe (lpm) | $k_La$ | RPM | Frit Flow (lpm) | Open Pipe (lpm) | $k_La$ |
| 20 | 8 | 0 | 3.77 | 20 | 8 | 12 | 5.90 |
| 20 | 12 | 0 | 6.11 | 20 | 12 | 12 | 8.22 |
| 20 | 16 | 0 | 7.92 | 20 | 16 | 12 | 10.01 |
| 20 | 20 | 0 | 9.19 | 20 | 20 | 12 | 11.26 |
| 20 | 24 | 0 | 9.92 | 20 | 24 | 12 | 11.97 |
| 35 | 8 | 0 | 3.94 | 35 | 8 | 12 | 6.23 |
| 35 | 12 | 0 | 6.41 | 35 | 12 | 12 | 8.66 |
| 35 | 16 | 0 | 8.33 | 35 | 16 | 12 | 10.57 |
| 35 | 20 | 0 | 9.72 | 35 | 20 | 12 | 11.94 |
| 35 | 24 | 0 | 10.58 | 35 | 24 | 12 | 12.77 |
| 55 | 8 | 0 | 5.61 | 55 | 8 | 12 | 8.08 |
| 55 | 12 | 0 | 8.23 | 55 | 12 | 12 | 10.68 |
| 55 | 16 | 0 | 10.31 | 55 | 16 | 12 | 12.74 |
| 55 | 20 | 0 | 11.86 | 55 | 20 | 12 | 14.27 |
| 55 | 24 | 0 | 12.87 | 55 | 24 | 12 | 15.26 |
| 75 | 8 | 0 | 5.98 | 75 | 8 | 12 | 8.65 |
| 75 | 12 | 0 | 8.76 | 75 | 12 | 12 | 11.41 |
| 75 | 16 | 0 | 11.01 | 75 | 16 | 12 | 13.63 |
| 75 | 20 | 0 | 12.71 | 75 | 20 | 12 | 15.31 |
| 75 | 24 | 0 | 13.88 | 75 | 24 | 12 | 16.46 |
| 95* | 8 | 0 | 6.77 | 95* | 8 | 12 | 9.63 |
| 95* | 12 | 0 | 9.71 | 95* | 12 | 12 | 12.55 |
| 95* | 16 | 0 | 12.11 | 95* | 16 | 12 | 14.93 |
| 95* | 20 | 0 | 13.98 | 95* | 20 | 12 | 16.77 |
| 95* | 24 | 0 | 15.31 | 95* | 24 | 12 | 18.08 |

**Table 6.6** Results for 2,000L S.U.B.

***NOTE:** Standard operating range is 20 to 75rpm. Custom operating limit up to 95rpm at 90 to 110% working volume only.




**Graph 6.4** Results for 2,000 L S.U.B.

*For Rotary Vane Air Motor Maintenance Kits, go to Grainger.com®*

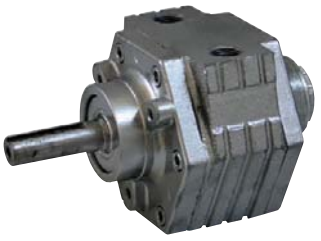
SPEEDAIRE®

# Rotary Vane Air Motors

- Max. temp.: 250°F
- Variable speed

Use these compact, low-maintenance air motors in applications that would normally require similar, but more-expensive explosionproof electric motors. Can be operated in extreme temperatures. Cast-iron models include discharge muffler.

No. 22UX55



No. 22UX45

No. 22UX58

| HP | Running Torque @ Max. Pressure | Max. Air Flow | No Load RPM | Shaft Dia. | Mounting | Port Size | Rotation* | Item No. |
|---|---|---|---|---|---|---|---|---|
| **Cast-Iron Air Gearmotors** | | | | | | | | |
| 0.32 | 49.00 in.-lb. | 21 cfm | 400 | ½" | Face | ⅛" NPT | REV | **22UX55** |
| 0.34 | 62.00 in.-lb. | 21 cfm | 350 | ½" | Face | ⅛" NPT | REV | **22UX82** |
| 0.64 | 172.00 in.-lb. | 20 cfm | 110 | ⅞" | Base | ¼" NPT | CCW | **22UX56** |
| 0.9 | 256.00 in.-lb. | 27 cfm | 150 | ⅝" | Base | ¼" NPT | CCW | **22UX57** |
| 1.25 | 400.00 in.-lb. | 60 cfm | 200 | 1" | Face and Foot | ¼" NPT | REV | **22UX81** |
| 1.26 | 274.00 in.-lb. | 57.5 cfm | 300 | 1" | Face and Foot | ¼" NPT | REV | **22UX54** |
| **Cast-Iron Air Motors** | | | | | | | | |
| 0.45 | 2.75 in.-lb. | 20.5 cfm | 10,000 | ⅜" | Hub | ⅛" NPT | CCW | **22UX71** |
| | 5.25 in.-lb. | 27 cfm | 6000 | ⅜" | Hub | ⅛" NPT | CW | **22UX41** |
| | 5.25 in.-lb. | 27 cfm | 6000 | ⅜" | Hub | ⅛" NPT | REV | **22UX42** |
| 0.93 | 19.50 in.-lb. | 30 cfm | 3000 | ½" | Hub | ¼" NPT | CCW | **22UX43** |
| | 19.50 in.-lb. | 30 cfm | 3000 | ⅝" | Flange | ¼" NPT | REV | **22UX44** |
| | 19.50 in.-lb. | 30 cfm | 3000 | ½" | Foot | ¼" NPT | REV | **22UX72** |
| 1.7 | 36.00 in.-lb. | 78 cfm | 3000 | ⅝" | Face | ⅜" NPT | REV | **22UX45** |
| | 36.00 in.-lb. | 78 cfm | 3000 | ⅝" | Flange | ¼" NPT | REV | **22UX46** |
| | 36.00 in.-lb. | 78 cfm | 3000 | ½" | Foot | ¼" NPT | REV | **22UX73** |
| | 36.00 in.-lb. | 78 cfm | 3000 | ½" | Foot | ¼" NPT | REV | **22UX74** |
| | 36.00 in.-lb. | 78 cfm | 3000 | ½" | Face | ¼" NPT | REV | **22UX75** |
| 4 | 84.00 in.-lb. | 128 cfm | 3000 | ⅝" | Foot | ½" NPT | REV | **22UX47** |
| | 84.00 in.-lb. | 128 cfm | 3000 | ⅝" | Flange | ½" NPT | REV | **22UX48** |
| | 84.00 in.-lb. | 128 cfm | 3000 | ⅝" | Flange | ½" NPT | REV | **22UX49** |
| | 84.00 in.-lb. | 128 cfm | 3000 | ⅝" | Foot | ½" NPT | REV | **22UX76** |
| | 84.00 in.-lb. | 128 cfm | 3000 | ⅝" | Face | ½" NPT | REV | **22UX77** |
| 5.25 | 132.00 in.-lb. | 175 cfm | 2500 | ¾" | Foot | ½" NPT | REV | **22UX50** |
| | 132.00 in.-lb. | 175 cfm | 2500 | ⅞" | Flange | ½" NPT | REV | **22UX51** |
| 9.5 | 290.00 in.-lb. | 275 cfm | 2000 | 1⅛" | Face and Foot | 1¼" NPT | REV | **22UX52** |
| | 290.00 in.-lb. | 275 cfm | 2000 | ⅞" | Foot and Flange | 1¼" NPT | REV | **22UX53** |
| **Cast-Iron Oilless Air Motors** | | | | | | | | |
| 0.42 | 13.50 in.-lb. | 30 cfm | 2000 | ½" | Hub and Foot | ¼" NPT | CCW | **22UX58** |
| | 13.50 in.-lb. | 30 cfm | 2000 | ½" | Hub and Foot | ¼" NPT | CW | **22UX59** |
| 0.82 | 25.50 in.-lb. | 41 cfm | 2000 | ½" | Hub and Foot | ¼" NPT | CCW | **22UX60** |
| 2.5 | 78.75 in.-lb. | 68 cfm | 2000 | ⅝" | Hub and Foot | ½" NPT | CW | **22UX63** |
| | 78.75 in.-lb. | 68 cfm | 2000 | ⅝" | Hub and Foot | ½" NPT | CCW | **22UX62** |
| **Stainless Steel Air Motors** | | | | | | | | |
| 0.45 | 2.75 in.-lb. | 20.5 cfm | 10,000 | ⅜" | Face | ⅛" NPT | REV | **22UX65** |
| 1.7 | 36.00 in.-lb. | 78 cfm | 3000 | ⅜" | Face | ¼" NPT | REV | **22UX67** |

** Clockwise (CW), Counterclockwise (CCW), Reversible (REV).*

Ingersoll Rand

# Rotary Multivane Air Gearmotors

- Max. air pressure: 90 psi
- Max. temp.: 150°F

Provide higher torque at low speeds and continuous-duty forward or reverse operation. Keyed steel shafts with case-hardened gearing. Aluminum and steel construction.

No. 1AAG1

| HP | Running Torque @ Max. Pressure | Stall Torque | Max. Air Flow | No Load RPM | RPM @ Max. Torque | Shaft Dia. | Shaft Length | Keyway Length | Port Size | Gear Ratio | Overall Length | Outside Dia. | Mfr. Model | Item No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Flange** | | | | | | | | | | | | | | |
| 0.31 | 99.00 in.-lb. | 198 in.-lb. | 18 cfm | 280 | 140 | ⅜" | 1.00" | 0.125" | ¼" NPT | 50:1 | 8.87" | 1.76" | M004RVR050AR3 | **1AAF9** |
| | 330.00 in.-lb. | 396 in.-lb. | 18 cfm | 85 | 42 | ⅜" | 1.00" | 0.125" | ¼" NPT | 167:1 | 8.87" | 1.76" | M004RVR167AR3 | **1AAG1** |
| 0.34 | 71.00 in.-lb. | 142 in.-lb. | 18 cfm | 450 | 225 | ⅜" | 1.00" | 0.125" | ¼" NPT | 33:1 | 8.11" | 1.76" | M004RVR033AR3 | **1AAF8** |
| **Face** | | | | | | | | | | | | | | |
| 0.61 | 612.00 in.-lb. | 1224 in.-lb. | 33 cfm | 94 | 47 | ¾" | 2.19" | 0.187" | ¼" NPT | 188:1 | 13.13" | 2.69" | M007RVR188BR6 | **1AAG2** |
| | 1212.00 in.-lb. | 2424 in.-lb. | 33 cfm | 47 | 23 | ¾" | 2.19" | 0.187" | ¼" NPT | 374:1 | 13.13" | 2.69" | M007RVR374BR6 | **1AAG3** |
| 3 | 1800.00 in.-lb. | 3600 in.-lb. | 95 cfm | 170 | 83 | 1" | 2.25" | 0.250" | ½" NPT | 51:1 | 14.50" | 4.00" | 4840U | **1AAG5** |
| 3.2 | 378.00 in.-lb. | 756 in.-lb. | 95 cfm | 975 | 485 | 1" | 2.25" | 0.250" | ½" NPT | 9:1 | 12.13" | 4.00" | 4840M | **1AAG4** |

GAST
A UNIT OF IDEX CORPORATION

# Tank-Mounted, Enclosed Oilless Air Compressors

- Field-adjustable pressure switch
- Single phase

Innovative reservoir can provide a blast of high pressure. Tank dampens pulsation from the reciprocating compressor.

All models except No. 5Z671 incorporate a Gast piston compressor pump listed below. Pressure switch setting: 70 psi-On/90 psi-Off. No. 5Z671 has 30 psi-On/50 psi-Off. Gray finish.

**⅛- and 1/6-HP Compressors—**Include pressure switch, manual drain, pressure safety valve, pressure gauge, and globe valve.

**⅓- to 1½-HP Compressors—**Include coded tank, pressure switch, manual drain, pressure safety valve, pressure gauge, and globe valve. No. 5Z676 has magnetic starter.

No. 5Z672

No. 5Z675

| HP | Free Air CFM @ Max. Pressure | Max. Pressure | Voltage | Amps AC | Tank Size | (F)NPT Outlet | Overall L | Overall W | Overall H | Mfr. Model | Item No. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ⅛ | 0.2 | 50 psi | 115 | 4.2 | 2 gal. | ¼" | 18" | 8" | 15" | DOA-P710T-AA | **5Z671** |
| | 0.4 | 100 psi | 115 | 4.3 | 2 gal. | ¼" | 18" | 8" | 17" | 1HAB-84T-M100X | **5Z672** |
| 1/6 | 0.9 | 50 psi | 115 | 3.9 | 2 gal. | ¼" | 18" | 8" | 17" | 1LAA-251T-M100X | **2CJH1** |
| ⅓ | 1.1 | 100 psi | 115 | 6.6 | 12 gal. | ¼" | 26" | 14" | 21" | 3HBB-69T-M300AX | **11X372** |
| ¾ | 4.7 | 100 psi | 115/230 | 15.8/7.9 | 2 gal. | ¼" | 18" | 13" | 22" | 5HCD-101T-M550NGX | **5Z674** |
| | 4.7 | 100 psi | 115/230 | 15.8/7.9 | 20 gal. | ¼" | 33" | 16" | 27" | 5HCD-100TA-M550NGX | **5Z675** * |
| 1½ | 5 | 100 psi | 230 | 10.8 | 30 gal. | ⅜" | 38" | 17" | 29" | 7HDD-70TA-M750X | **5Z676** |

** No. 5Z675 is factory wired for 230V, may be converted to 115V.*

Jenny

# Fire Sprinkler Air Compressors and Pumps

- 115/230V

Single stage with durable powder-coated finish.

**Fire Sprinkler Air Compressors—**Single-stage, splash-lubricated compressors are rugged cast-iron construction with a large flywheel for extra cooling and to ease start-up. Feature a directional air shroud to reduce pump temp., large canister intake filter with replaceable filter elements, totally enclosed heavy-duty belt guard, and an open dripproof motor with thermal overload protection.

- Max. pressure: 40 psi

**Air Compressor Pumps—**Cast-iron pumps have stainless steel valves and use synthetic oil for longer life. No. 35KV12 has head unloaders.

- Max. pressure: 150 psi

No. 31LC92


No. 31LC99

| HP | Free Air CFM @ Max. Pressure | Phase | Amps | Tank Size | (F)NPT Outlet | Overall Length | Overall Width | Overall Height | Mfr. Model | Item No. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Fire Sprinkler Air Compressors** | | | | | | | | | | |
| **Base Mounted** | | | | | | | | | | |
| 0.50 | 3.80 | 1 | 9.4/4.7 | — | ½" | 25¾" | 16¼" | 18¼" | F12S-BS-115/1-ACGF | **31LC94** |
| 1.00 | 7.40 | 1 | 12.5/6.3 | — | ½" | 25¾" | 16¼" | 18¼" | K1S-BS-115/1-ACGK | **31LC95** |
| 1.50 | 10.9 | 1 | 15.8/7.9 | — | ½" | 25¾" | 16¼" | 18¼" | K15S-BS-115/1-ACGK | **31LC96** |
| 2.00 | 12.4 | 1 | 21.6/10.8 | — | ½" | 25¾" | 16¼" | 18¼" | K2S-BS-115/1ACGK | **31LC97** |
| **Tank Mounted** | | | | | | | | | | |
| 0.50 | 3.70 | 1 | 9.4/4.7 | 30 gal. | ½" | 40" | 18" | 33½" | F12S-30UMS-115/1 | **26JY13** |
| 1.00 | 7.40 | 1 | 12.5/6.3 | 30 gal. | ½" | 40" | 18" | 34" | K1S-30UMS-115/1 | **31LC92** |
| 1.50 | 10.9 | 1 | 15.8/7.9 | 30 gal. | ½" | 40" | 18" | 34" | K15S-30UMS-115/1 | **31LC93** |
| 2.00 | 10.5 | 1 | 21.3/10.7 | 30 gal. | ½" | 40" | 18" | 34" | K2S-30UMS-115/1 | **26JY14** |

| HP | Free Air CFM @ Max. Pressure | Pump RPM | Sheave Size @ 1725 RPM | Oil Cap. | NPT Outlet | Flywheel Dia. | Groove Section | Groove Quantity | Mfr. Model | Item No. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Air Compressor Pumps** | | | | | | | | | | |
| ⅓, 1 | 2.1 | 1200 | 6²¹⁄₆₄" | 8 oz. | ½" | 10⅛" | A | 1 | F-PUMP | **31LC98** |
| 1, 2 | 6.7 | 1200 | 6²¹⁄₆₄" | 32 oz. | ½" | 10⅛" | A | 1 | K-PUMP | **31LC99** |
| | 6.7 | 1200 | 6²¹⁄₆₄" | 32 oz. | ½" | 10⅛" | A | 1 | KU-PUMP | **35KV12** * |

***Has head unloaders.***

No. 4B242

# Base-Plate Air Compressors

SPEEDAIRE

- Designed to cycle On/Off while providing continuous air with an 80/20 duty cycle
- All compressors must be hardwired
- Max. pressure: 40 psi

2-cylinder cast-iron pumps can be used in many fire sprinkler applications (check local codes and regulations). Prewired (240V) with pneumatic control package.

| HP | Free Air CFM @ Max. Pressure | Phase | Input Voltage | Amps | Motor Starter No. | Thermal Unit Code | (F)NPT Outlet | Overall Length | Overall Width | Overall Height | Item No. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.00 | 11.6 | 1 | 120VAC, 240VAC | 17.5/8.75 | — | — | ½" | 22½" | 15½" | 14¾" | **4B242** |
| 3.00 | 12.3 | 3 | 200 to 240VAC, 480VAC | 9.0-7.5/3.9 | 11V709, 11V710 | 1H609/3, 1H632/3 | ½" | 22½" | 15½" | 14¾" | **4B243** |

No. 5Z702

| HP | RPM | Pump Cylinders | Bore | Stroke | Oil Cap. (oz.) |
|---|---|---|---|---|---|
| ¾* | 980 | 1 | 1.97 | 2.56 | 15 |
| 1 | 1120 | 1 | 1.97 | 2.56 | 15 |
| 1½ | 860 | 1 | 2.56 | 2.56 | 15 |
| 3 | 1050 | 2 | 2.56 | 2.36 | 24 |

| HP | Free Air CFM @ Max. Pressure | Tank Size | Input Voltage | Amps | Phase | Overall Length | Overall Width | Overall Height | Item No. |
|---|---|---|---|---|---|---|---|---|---|
| **Simplex** | | | | | | | | | |
| 0.75 | 2.1 | 30 gal. | 120VAC, 240VAC | 10.2/5.1 | 1 | 42½" | 21¼" | 38" | **5Z696** |
| | 2.1 | 30 gal. | 208 to 240VAC, 480VAC | 3.6-3.0/1.5 | 3 | 42½" | 21¼" | 38" | **5Z697** |
| 1.00 | 3.4 | 30 gal. | 120VAC, 240VAC | 13.4/6.7 | 1 | 42½" | 21¼" | 38" | **3JR83** |
| 1.50 | 4.5 | 60 gal. | 208 to 240VAC, 480VAC | 4.5-4.4/2.2 | 3 | 50¾" | 22¼" | 40" | **5Z699** |
| 3.00 | 10.1 | 80 gal. | 208 to 240VAC, 480VAC | 8.9-8.1/4.1 | 3 | 65½" | 23¼" | 34" | **5Z701** |
| **Duplex** | | | | | | | | | |
| 0.75 | 4.2 | 30 gal. | 208 to 240VAC, 480VAC | 7.4-6.2/3.1 | 3 | 41½" | 21¼" | 38" | **5Z698** |
| 1.00 | 6.8 | 30 gal. | 208 to 240VAC, 480VAC | 6.4-6.2/3.1 | 3 | 41½" | 21¼" | 38" | **3JR81** |
| 1.50 | 9 | 80 gal. | 208 to 240VAC, 480VAC | 9.3-9.0/4.5 | 3 | 64¼" | 22¼" | 39" | **5Z700** |
| 3.00 | 11.0 | 80 gal. | 208 to 240VAC, 480VAC | 18.2-16.5/8.2 | 3 | 65¼" | 23¼" | 34" | **5Z702** |

***For altitudes over 5000 ft., flow will be reduced; select 1 HP instead.***

SPEEDAIRE

# Climate Control Air Compressors

- 3-phase duplex units require 115V for control panel power
- Max. pressure: 90 psi
- Simplex: 70 psi On/90 psi Off
- Duplex: 70 psi On/90 psi Off (lead), 60 psi On/80 psi Off (lag)
- All compressors must be hardwired
- Horizontal tank with ¾" (F)NPT outlet and brass ball valves

Deliver published cfm at stated rpm and pressure and to be below 2 ppm in oil carryover. Use for new, replacement, or upgrade HVAC installations and instrument air applications. Simplex packages include mounted and wired magnetic starter (except 1-phase); duplex packages include mounted and wired alternator and 2 magnetic starters. For duplex models, use twice the oil capacity (Compressor Oil No. 4ZF21 is recommended, sold separately on page 2568).

No. 39CC74

No. 39CC68

No. 39CC70

# Mobile Engine-Driven Air Compressors and Compressor/Generator

VANAIR MOBILE POWER SOLUTIONS

Rotary screw-type with electric start. Include a lifetime manufacturer's warranty on the compressor and are Tier 4 compliant.

**Viper Air Compressors—**Can easily power a 90-lb. jackhammer and include a 12V cold weather protection kit. Diesel models feature an automatic variable throttle control to help reduce fuel consumption.

**Viper Air Compressor/Generator—**Features multi-functioning capabilities and provides air and AC power in 1 portable unit. Can run at full load for up to 4 hr. and is easy to transport via fork lift or pallet truck.

**PowerFlex™ Air Compressor/Generator—**Diesel-powered model provides air and AC power in one easily transportable unit. Includes a 12V cold weather protection kit.

**Air N Arc® Compressor/Generator/Welder/Battery Charge System—**Multifunction systems include a 12V cold weather protection kit and 25-ft. weld and boost cables. Battery boost cables are available on Grainger.com®.

| Description | Free Air CFM @ Max. Pressure | Max. Pressure | (F)NPT Outlet | Rated Watts | Fuel Type | Fuel Tank Capacity | Overall Length | Overall Width | Overall Height | Weight | Item No. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Viper Air Compressors** | | | | | | | | | | | |
| Viper G60 | 60.0 | 150 psi | ¾" | — | Gasoline | 5.00 gal. | 45³²⁄₃₂" | 23½" | 29¾" | 480 lb. | **39CC67** |
| Viper G80 | 59.0 | 100 psi | ¾" | — | Gasoline | 5.00 gal. | 45³²⁄₃₂" | 23½" | 29¾" | 480 lb. | **39CC74** |
| Viper D60 | 60.0 | 150 psi | ¾" | — | Diesel | 9.00 gal. | 48" | 21" | 33" | 800 lb. | **39CC75** |
| Viper D80 | 80.0 | 100 psi | ¾" | — | Diesel | 9.00 gal. | 47" | 24" | 38" | 800 lb. | **39CC71** |
| **Viper Air Compressor/Generator** | | | | | | | | | | | |
| — | 40.0 | 100 psi | ¾" | 5000/5200 | Gasoline | 5.00 gal. | 42" | 33" | 34½" | 685 lb. | **4TZW4** |
| **PowerFlex™ Air Compressor/Generator** | | | | | | | | | | | |
| Powerflex AE | 40.0 | 150 psi | ¾" | 7000 | Diesel | 12.0 gal. | 47³⁹⁄₆₄" | 21" | 33¹³⁄₃₂" | 803 lb. | **39CC70** |
| **Air N Arc® Compressor/Generator/Welder/Battery Charge System** | | | | | | | | | | | |
| Air N Arc® 300 Gas | 40.0 | 175 psi | ¾" | 7000 | Gasoline | 12.0 gal. | 47" | 21" | 31" | 700 lb. | **39CC68** |
| Air N Arc® 300 Diesel | 40.0 | 175 psi | ¾" | 7000 | Diesel | 12.0 gal. | 47" | 21" | 33" | 865 lb. | **39CC73** |

# Clarification Portfolio Guide

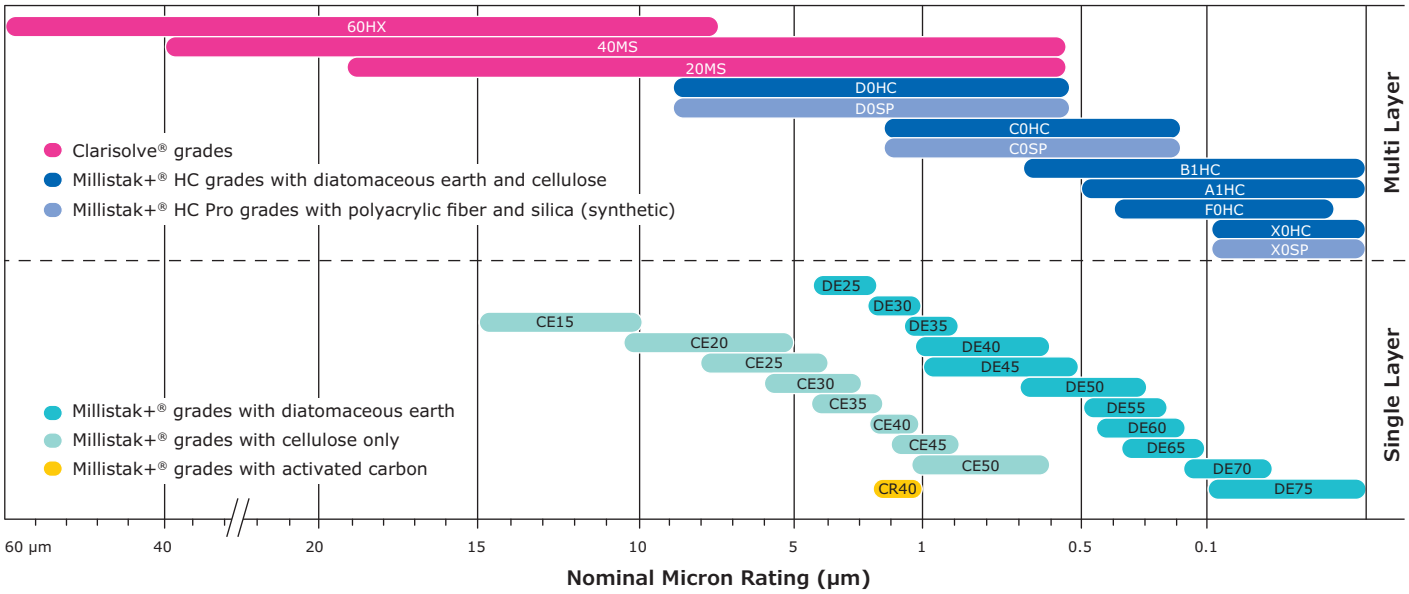
Single- and multi-use products for the successful development and implementation of robust clarification processes

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Millipore®

Preparation, Separation, Filtration & Monitoring Products

# Media Grades




| | | Target Step | Media Grade |
|---|---|---|---|
| Clarisolve® | Pre-treated Feed | Single stage clarification of mAB pre-treated feed streams with particle size distribution of 60 µm (Cationic Polymers such as Clarisolve® mPAA). Utility in some bacterial, viral and polysaccharide vaccine applications. | **60HX** |
| | | Single stage clarification of mAb pre-treated feed streams with particle size distribution of 40 µm (Cationic Polymers such as pDADMAC). Utility in some bacterial and viral vaccine applications. | **40MS** |
| | | Single stage clarification of mAb pre-treated feed streams with particle size distribution of 20 µm (Acid Precipitation). Utility in some bacterial and viral vaccine applications. | **20MS** |
| Millistak+®, Millistak+® HC, Millistak+® HC Pro | Primary Clarification | Primary clarification. In some cases, can be used as a single stage clarification step going directly into a sterile filter. | **C0HC**<br>**C0SP (synthetic)** |
| | | Primary (coarse) clarification | **CE** |
| | | Primary clarification | **D0HC**<br>**D0SP (synthetic)** |
| | | Primary or secondary clarification | **DE** |
| | Secondary Clarification | Secondary clarification post tangential flow filtration or depth filtration | **A1HC** |
| | | Secondary clarification post-centrifugation | **B1HC** |
| | | Secondary clarification post–centrifugation or pre-treated feed streams | **F0HC** |
| | | Secondary clarification post depth filtration or centrifugation. Can also be utilized in downstream processing steps to protect chromatography columns. | **X0HC**<br>**X0SP (synthetic)** |
| | DSP | Removal of color and trace contaminants, as well as in downstream processing to reduce HCP and other impurities present in Protein A elution pools. | **Carbon (AC) CR40**<br>**X0SP (synthetic)** |

# Device Formats

## Pod Filter Systems (Millistak+® and Clarisolve® media)

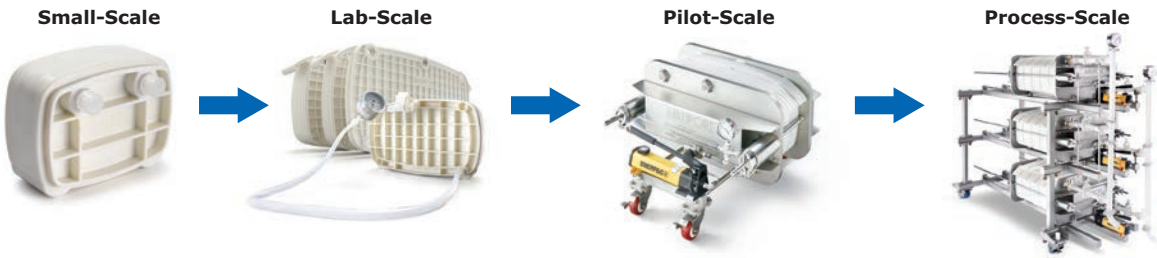
The Pod Filter System is a scalable format accommodating applications from lab- to pilot- to process-scale. The Pod format offers flexibility because of its unique, modular and 100% disposable design. The same flow path and configuration ensures a linearly scalable solution from bench- to process-scale.

- Patented, disposable design eliminates need for housing, CIP or cleaning validation
- Self-contained Pod format protects operators from exposure to biohazards
- Enables use of multiple grades in one holder
- Robust construction is easy to use and set up
- Smaller footprint facilitates use in tight spaces

The Pod holder system's modular design makes it easy to configure for a specific application and conveniently reconfigure it as process capacity requirements scale-up or -down. The flexible, modular format offers scalability up to 12,000 L or more.

Ordering Information

Virtual Pod Tool




## Lenticular Discs (Millistak+® media only)

The Millistak+® 16 in. lenticular format is available in multiple stacked-cell configurations. The individual cells of filter media are combined to form a convenient and easy-to-install filtration unit. Each filter cell is independently sealed by an injection molding process to ensure integrity throughout each device. Edge seal bosses provide robustness against cell collapse during prolonged runs, minimizing the risk of bypass and process deviations.

The Millistak+® 316 L stainless steel filter housings are designed for high-capacity liquid clarification. The housings' versatile design and a wide offering of accessories allow users to operate the system with as few as one or as many as eight stacked cartridges installed. These options make it easy to configure for a specific application and conveniently reconfigure it as process capacity requirements scale-up or -down.

Ordering Information

# Clarification Selection Guide for Monoclonal Antibodies, Recombinant Proteins, and Fc-fusion Proteins

The following decision trees are focused on batch and fed-batch feeding strategies for mammalian cell bioreactors. The selection matrices are created for reference only, based on historical experience and data.

The decision trees cover direct harvest, pretreatment, and centrifugation at various scales. Studies have shown that direct harvest is more economical versus centrifuge at scales below 1000 L*. There is a “grey area” between 1000 L and 2000 L where either direct harvest or centrifuge may be the best choice, depending on cell culture characteristics and facility considerations.

## Direct Harvest Decision Tree

### Molecule Types: mAb, recombinant, fusion, other-hydrophobic proteins

Monoclonal antibodies, recombinant proteins, and Fc-fusion proteins behave similarly with respect to capacity. Filters containing diatomaceous earth may not be the ideal solution for Fc-fusion proteins and highly hydrophobic proteins, due to recovery challenges. If product yield is an issue, reach out to your local Account Manager or Process Development Specialist.

Two filtration options are included:

1. A single-stage depth filtration train.
2. A two-stage depth filtration train run in series.

The single-stage train is recommended in cases of low-titer feeds and/or when there are concerns about product binding.

The information in the following tables should be considered as general guidance for performance and preliminary economic assessments. Actual performance should be confirmed prior to large-scale implementation.

Please note: The single filter will need to be optimized for both capacity and filtrate turbidity/sterile filter capacity. The second filter in the filter train is tight enough to protect the sterile filter in most cases. Secondary depth filtration is included for additional sterile filter and column protection in most cases.

*Process Cost and Facility Considerations in the Selection of Primary Cell Culture Clarification Technology, Felo et al., Biotechnol. Prog., 2013, Vol. 29, No. 5.

| **Cell Density** | ≤ $10E^6$ cells/mL (low) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Cell Viability*** | Low Density/Low Viability | | | | Low Density/High Viability | | | |
| **Depth Filter Train** | Single-stage | | Two-stage | | Single-stage | | Two-stage | |
| **Millistak+® Media Grade** | C0HC | HC Pro C0SP† | D0HC/X0HC | HC Pro D0SP/X0SP† | C0HC | HC Pro C0SP† | D0HC/X0HC | HC Pro D0SP/X0SP† |
| **Filter Loading** | 150 L/m² | 300 L/m² | D0HC: 50 L/m² X0HC: 150 L/m² | D0SP: 100 L/m² X0SP: 150 L/m² | 125 L/m² | 250 L/m² | D0HC: 50 L/m² X0HC: 250 L/m² | D0SP: 100 L/m² X0SP: 250 L/m² |
| Scale‡ 1 L |  µPod® device format | | | | | | | |
| 10 L |  Lab-scale pod (LSP) device format | | | | | | | |
| 100 L<br>1000 L<br>2000 L |  Process-scale pod (PSP) device format | | | | | | | |
| 10,000 L | see centrifuge | | | | | | | |

| **Cell Density** | 20–$30E^6$ cells/mL (high) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Cell Viability*** | High Density/Low Viability | | | | High Density/High Viability | | | |
| **Depth Filter Train** | Single-stage | | Two-stage | | Single-stage | | Two-stage | |
| **Millistak+® Media Grade** | C0HC | HC Pro C0SP† | D0HC/X0HC | HC Pro D0SP/X0SP† | C0HC | HC Pro C0SP† | D0HC/X0HC | HC Pro D0SP/X0SP† |
| **Filter Loading** | 100 L/m² | 200 L/m² | D0HC: 50 L/m² X0HC: 150 L/m² | D0SP: 100 L/m² X0SP: 150 L/m² | 75 L/m² | 150 L/m² | D0HC: 25 L/m² X0HC: 250 L/m² | D0SP: 100 L/m² X0SP: 250 L/m² |
| Scale‡ 1 L |  µPod® device format | | | | | | | |
| 10 L |  Lab-scale pod (LSP) device format | | | | | | | |
| 100 L<br>1000 L<br>2000 L |  Process-scale pod (PSP) device format | | | | | | | |
| 10,000 L | see centrifuge | | | | | | | |

Note: If the filtrate turbidity is greater than 15–20 NTU or sterile sizing does not meet your target directly after the primary depth filter, consider additional polishing via X0HC.

Protein titer (<1 g/L, >1 g/L). Recovery should be considered during the small-scale tests. Low-titer feeds historically have slightly lower recovery values. This could be due to low levels of binding, hold-up volume losses during recovery (blow-down or buffer flush), and/or dilution from pre-use flushing and post-use recovery.

*Cell density is a critical parameter in the consideration for filter selection and capacity. High-cell-density cultures contain a high level of cells and with that cell debris, both soluble and insoluble. When the viability is high, most of the cells are intact and the particle size distribution shifts towards larger particles. When the viability is low, the particle size shifts towards the smaller particles and insoluble (unmeasured) particles.

† As a general guideline, the filter loadings for Millistak+® HC Pro Media grades (D0SP and C0SP only) are at least double (2X) the equivalent Millistak+® HC D0HC and C0HC grades. The X0 grades for both have similar filter loadings.

‡ Process scales are typically 1 L–20,000 L. For simplicity, the process scale increases by 10x starting with 1 L. Direct harvest is economically practical and feasible for manufacturing, depending on the cell density, up to approximately 2,000 L. Centrifuge is recommended at scales ≥2,000 L for direct harvest.

## Pretreatment Decision Tree

To meet the technical challenges of high titer mammalian cell culture processes, our novel chemical flocculants can be used to pretreat high density cell harvests before clarification over Clarisolve® depth filters. Pretreatment, via flocculation or precipitation, is recommended at cell densities greater than or equal to $30\times10^6$ cells/mL. However, flocculation can be implemented at any cell density if the process requires such a step. The type of pretreatment and dosing requirements (e.g., pH adjustment, salt addition, polymer addition) depends on the cell culture and molecule characteristics. The Clarisolve® family of filters was developed for high-cell-density, pretreated feed streams. The filter selection and sizing recommendations will be based on the type of pretreatment and the particle size distribution of the pretreated feed stream.

| **Cell Density** | **> 30E⁶ cells/mL (volumes ≤ 5000L)** | | | |
|---|---|---|---|---|
| | ↓ | ↓ | ↓ | ↓ |
| | **Acid treatment** | **Salt addition** | **pDADMAC cationic polymer** | **Clarisolve® mPAA stimulus responsive cationic polymer** |
| Clarisolve® 20MS* filter | 1st choice | 2nd choice | 2nd choice | 3rd choice |
| Clarisolve® 40MS* filter | 2nd choice | 1st choice | 1st choice | 2nd choice |
| Clarisolve® 60HX* filter | — | — | 3rd choice | 1st choice |

*If the filtrate turbidity is greater than 15–20 NTU or sterile sizing does not meet your target directly after the primary depth filter, consider additional polishing via X0HC or XOSP.

## Centrifuge Decision Tree

Centrate may differ depending on the cell density/viability and the centrifuge optimization/operation. Centrate turbidity is a good indicator as to which depth filter will have the highest capacity and best sterile filter protection.

| **Cell Density** | **< 30E⁶ cells/mL (low) untreated** | | **> 30E⁶ cells/mL (high) pretreated** | |
|---|---|---|---|---|
| | ↓ | | ↓ | |
| | **High centrate turbidity (>100 NTU)** | **Low centrate turbidity (<100 NTU)** | **High centrate turbidity (>100 NTU)** | **Low centrate turbidity (<100 NTU)** |
| **1000 L** | | | | |
| Millistak+® filter, F0HC media series (1st choice) B1HC (2nd choice) | Typical loading: 200–400 L/m² Device format: Process-scale pod | — | Typical loading: 200–400 L/m² Device format: Process-scale pod | — |
| Millistak+® filter, X0HC/X0SP media series (1st choice) A1HC (2nd choice) | — | Typical loading: 200–400 L/m² Device format: Process-scale pod | — | Typical loading: 200–400 L/m² Device format: Process-scale pod |
| | ↓ | ↓ | ↓ | ↓ |
| **2000 L** | | | | |
| Millistak+® filter, F0HC media series (1st choice) B1HC (2nd choice) | Typical loading: 200–400 L/m² Device format: Process-scale pod | — | Typical loading: 200–400 L/m² Device format: Process-scale pod | Typical loading: 200–400 L/m² Device format: Process-scale pod |
| Millistak+® filter, X0HC/X0SP media series (1st choice) A1HC (2nd choice) | — | Typical loading: 200–400 L/m² Device format: Process-scale pod | Typical loading: 200–400 L/m² Device format: Process-scale pod | Typical loading: 200–400 L/m² Device format: Process-scale pod |
| | ↓ | ↓ | ↓ | ↓ |
| **10,000 L** | | | | |
| Millistak+® filter, F0HC media series (1st choice) B1HC (2nd choice) | Typical loading: 200–400 L/m² Device format: Process-scale pod | Typical loading: 200–400 L/m² Device format: Process-scale pod | Typical loading: 200–400 L/m² Device format: Process-scale pod | — |
| Millistak+® filter, X0HC/X0SP media series (1st choice) A1HC (2nd choice) | — | Typical loading: 200–400 L/m² Device format: Process-scale pod | Typical loading: 200–400 L/m² Device format: Process-scale pod | Typical loading: 200–400 L/m² Device format: Process-scale pod |

# Non-mAb Expression Systems Decision Tree

The following decision tree is focused on non-mAb expression systems at all scales. Non-mAb expression systems typically require more process development due to processing differences. Particle size distribution can vary greatly between molecules/processes. The recommendations are based on historical experience and data.

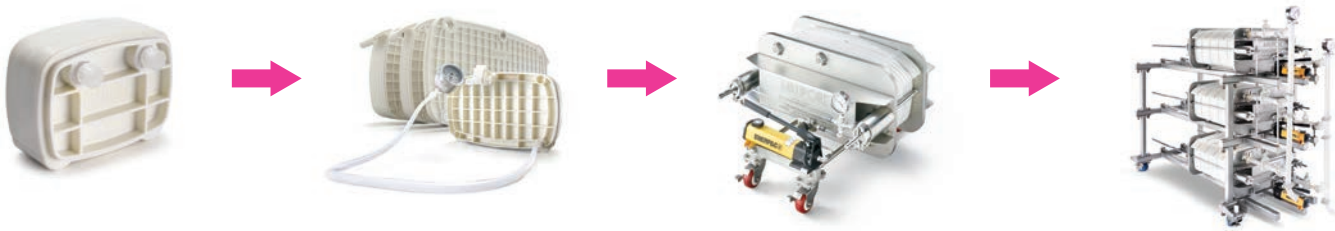
| Expression System | Molecule Type | Application | Batch Type | Pretreatment Options/ Method of Cell Lysis | Primary Clarification | Secondary Clarification |
|---|---|---|---|---|---|---|
| Mammalian | Enzyme (binds to DE) | Harvest | Perfusion | NONE | Millistak+® CE50 | Polysep™ II CGW6 |
| | | | | | Polygard® CR 0.5 µm | Polysep™ II CGW6 |
| | Enzyme (does not bind to DE) | Harvest | Perfusion | NONE | Millistak+® C0HC | Millistak+® X0HC |
| | | | | | Millistak+® F0HC | — |
| Microbial: Bacterial | Secreted Protein | Harvest | Batch | NONE | Millistak+® C0HC | Millistak+® X0HC |
| | | | | | Prostak™ 0.22 µm or 0.1 µm | — |
| | Intracellular—Soluble | Lysate Clarification | Batch | Mechanical | Pellicon® 2 1000 kD Biomax® V-screen | — |
| | | | | | Clarisolve® 20MS | Millistak+® C0HC |
| | | | | Chemical or Enzymatic | Call Technical Service | |
| | Intracellular—Inclusion Body | Lysate Clarification/ IB Wash | Batch | Mechanical | Prostak™ MF 0.1 µm | — |
| | | | | | Pellicon® 2 1000kD Biomax® V-screen | — |
| | | | | Chemical or Enzymatic | Call Technical Service | |
| | | Lysate Clarification/ Refold Pool | Batch | NONE | Clarisolve® 20MS | (Millistak+® X0HC - if needed)* |
| | | | | | Clarisolve® 60HX | (Millistak+® X0HC - if needed)* |
| Microbial: Yeast | Intracellular—Inclusion Body | Lysate Clarification | Batch | Mechanical | Clarisolve® 60HX | Millistak+® C0HC |
| | | | | | Pellicon® 2 1000kD Biomax® V-screen | — |
| Vaccines | Egg-based Influenza | Centrate Clarification | Batch | NONE | Polygard® CN 5.0 µm | — |
| | | | | | Polygard® CR 5.0 µm | — |
| | Cell-based Influenza | Centrate Clarification | Batch | NONE | Clarisolve® 20MS | — |
| | | | | | Polygard® CR 5.0 or 3.0 µm | Clarigard® 1.0 or 0.5 µm |
| | Viral Vector | Lysate Clarification | Batch | Mechanical | Clarigard® 3.0 µm | Polysep™ II CGW6 |
| | | | | | Pellicon® 2 0.65 µm Durapore® V-screen | — |
| | pDNA | Harvest Clarification | Batch | NONE | Pellicon® 2 0.1 µm Durapore® V-screen | — |
| | | | | | Pellicon® 2 1000kD Biomax® V-screen | — |
| | | Precipitate Clarification | Batch | NONE | Clarisolve® 60HX | — |
| | Virus-like Particle | Lysate Clarification | Batch | Mechanical | Prostak™ 0.65 µm Durapore® | — |
| | | | | | Clarisolve® 20MS | — |
| | | | | | Polygard® CN 5.0 µm | — |
| | Conjugated Polysaccharide | — | Batch | NONE | Prostak™ 0.1 µm Durapore® | — |

*If filtrate turbidity is greater than 15–20 NTU or sterile sizing does not meet your target directly after the primary depth filter, consider additional polishing via X0HC.

## Pod Hardware

| Description | | Qty/Pk | |
|---|---|---|---|
| Pilot-Scale Holder | For Pod configurations from 1 to 2 filters | 1 | **MP0DPIL0T** |
| | For Pod configurations up to 5 filters | 1 | **MP0DPILOTX** |
| Process-Scale Holder | 1-rack holder; Gemu® valves | 1 | **MP0DSYS1A** |
| | 1-rack holder; ITT valves | 1 | **MP0DSYS1B** |
| | 1-rack holder; no valves | 1 | **MP0DSYS1N** |
| | 1-rack expansion kit; no valves or casters | 1 | **MP0DSYS1X** |
| | 2-rack holder; Gemu® valves | 1 | **MP0DSYS2A** |
| | 2-rack holder; ITT valves | 1 | **MP0DSYS2B** |
| | 2-rack holder; no valves | 1 | **MP0DSYS2N** |
| | 3-rack holder; Gemu® valves | 1 | **MP0DSYS3A** |
| | 3-rack holder; ITT valves | 1 | **MP0DSYS3B** |
| | 3-rack holder; no valves | 1 | **MP0DSYS3N** |

**Small-Scale** **Lab-Scale** **Pilot-Scale** **Process-Scale**



## Disposable Adapter Kit[1]

| | |
|---|---|
| 3 through adapters, 3 blind adapters | **MP0DADAPT** |
| 6 through adapters, required if using Disposable Diverter Plate (MP0DDIVERTR) | **MP0DADPTF** |

[1]Note: Pod filters require the use of flow adaptors, which are sold separately (MP0DADAPT or MP0DADPTF). See the Millistak+® Pod disposable depth filter hardware Data Sheet (DS3388EN00) for information on Pod filter holders.

A retrofit kit may be required in order to accommodate the new Clarisolve® depth filters in the Pod pilot- and process-scale holders. Please contact your local sales representative for details.

## Millistak+® Lenticular Housings

| Number of Cartridges* | Inlet/Outlet Connections | Cat. No. |
|---|---|---|
| **CE Marked** | | |
| **Millistak+® Housings†** | | |
| **For 12 in. Diameter Millistak+® (DE, CE, A) Cartridges** | | |
| 1 x 12 in. 13- or 16-cell/6- or 9-cell | 1 in. TC, ISO DN32 | **WM21 SET ET** |
| 2 x 12 in. 13- or 16-cell/6- or 9-cell | 1 in. TC, ISO DN32 | **WM22 SET ET** |
| 3 x 12 in. 13- or 16-cell (4 x 12 in. 6- or 9-cell) | 1 in. TC, ISO DN32 | **WM23 SET ET** |
| 4 x 12 in. 13- or 16-cell (5 x 12 in. 6- or 9-cell) | 1 in. TC, ISO DN32 | **WM24 SET ET** |
| **For 16 in. Diameter Millistak+® (DE, CE, A) Cartridges** | | |
| 1 x 16 in. 16-cell | 2 in. TC, ISO DN40 | **WM61 SFT FT** |
| 2 x 16 in. 16-cell (3 x HC 16 in. 8-cell) | 2 in. TC, ISO DN40 | **WM62 SFT FT** |
| 3 x 16 in. 16-cell (4 x HC 16 in. 8-cell) | 2 in. TC, ISO DN40 | **WM63 SFT FT** |
| 4 x 16 in. 16-cell (6 x HC 16 in. 8-cell) | 2 in. TC, ISO DN40 | **WM64 SFT FT** |
| **Millistak+® HC Housings†** | | |
| **For 16 in. Diameter Millistak+® HC Cartridges** | | |
| 2 x 16 in. 8-cell (1 x DE, CE, A 16 in. 16-cell) | 2 in. TC, ISO DN40 | **HC62 SFT FT** |
| 4 x 16 in. 8-cell (2 x DE, CE, A 16 in. 16-cell) | 2 in. TC, ISO DN40 | **HC64 SFT FT** |
| 6 x 16 in. 8-cell (3 x DE, CE, A 16 in. 16-cell) | 2 in. TC, ISO DN40 | **HC66 SFT FT** |
| 8 x 16 in. 8-cell (5 x DE, CE, A 16 in. 16-cell) | 2 in. TC, ISO DN40 | **HC68 SFT FT** |
| **ASME® Stamped** | | |
| **Millistak+® Housings†** | | |
| **For 12 in. Diameter Millistak+® (DE, CE, A) Cartridges** | | |
| 1 x 12 in. 13- or 16-cell/6- or 9-cell | 1 in. TC | **UM21 SET ET** |
| 2 x 12 in. 13- or 16-cell/6- or 9-cell | 1 in. TC | **UM22 SET ET** |
| 3 x 12 in. 13- or 16-cell/4 x 12 in. 6- or 9-cell | 1 in. TC | **UM23 SET ET** |
| 4 x 12 in. 13- or 16-cell/5 x 12 in. 6- or 9-cell | 1 in. TC | **UM24 SET ET** |
| **For 16 in. Diameter Millistak+® (DE, CE, A) Cartridges** | | |
| 1 x 16 in. 16-cell | 2 in. TC | **UM61 SFT FT** |
| 2 x 16 in. 16-cell (3 x HC 16 in. 8-cell) | 2 in. TC | **UM62 SFT FT** |
| 3 x 16 in. 16-cell (4 x HC 16 in. 8-cell) | 2 in. TC | **UM63 SFT FT** |
| 4 x 16 in. 16-cell (6 x HC 16 in. 8-cell) | 2 in. TC | **UM64 SFT FT** |
| **Millistak+® HC Housings†** | | |
| **For 16 in. Diameter Millistak+® HC Cartridges** | | |
| 2 x 16 in. 8-cell (1 x DE, CE, A 16 in. 16-cell) | 2 in. TC | **UC62 SFT FT** |
| 4 x 16 in. 8-cell (2 x DE, CE, A 16 in. 16-cell) | 2 in. TC | **UC64 SFT FT** |
| 6 x 16 in. 8-cell (3 x DE, CE, A 16 in. 16-cell) | 2 in. TC | **UC66 SFT FT** |
| 8 x 16 in. 8-cell (5 x DE, CE, A 16 in. 16-cell) | 2 in. TC | **UC68 SFT FT** |

* Millistak+® Housings are also compatible with many other commercially available lenticular cartridges.

† The compression tool must be ordered separately (Compression kit assembly, Catalogue No.: 1 WM00 CAK 01).

# Prostak™ Microfiltration Modules

## Packaging: 1/pack

| Pore Size (µm) | 2-Stak | 4-Stak | 10-Stak | 20-Stak |
|---|---|---|---|---|
| **Microporous Membranes – Hydrophilic PVDF Durapore® Membrane** | | | | |
| 0.1 | **PSVV AG0 21** | **PSVV AG0 41** | **PSVV AG1 01** | **SK2P 127 E1** |
| 0.22 | **PSGV AG0 21** | **PSGV AG0 41** | **PSGV AG1 01** | **SK2P 484 E0** |
| 0.45 | **PSHV AG0 21** | **PSHV AG0 41** | **PSHV AG1 01** | **SK2P 242 E9** |
| 0.65 | **PSDV AGO 21** | **PSDV AG0 41** | **PSDV AG1 01** | **SK2P 446 E0** |
| **Microporous Membranes – Hydrophobic PVDF Durapore® Membrane** | | | | |
| 0.22 | — | — | — | **SK2P 344 W2** |
| 0.45 | **SK2P 012 W6** | — | — | **SK2P 013 W4** |
| **PZHK Membrane – Hydrophobic PVDF** | | | | |
| 200* | — | — | — | **SK2R B30 A1** |

*Nominal Molecular Weight Limit in kDaltons.
There is one module per package. Sanitary gaskets are supplied with each module to provide a leak-free connection between the module(s) and holder.

## Pellicon® 2 Filters with V Screens (loose screen)

| Membrane | 0.1 m²/1.1 ft² | 0.5 m²/5.4 ft² | 2.0 m²/21.5 ft² |
|---|---|---|---|
| **Biomax® Series – Modified Polyethersulfone** | | | |
| Biomax® 5 | P2B0 05V 01 | P2B0 05V 05 | P2B0 05V 20 |
| Biomax® 8 | P2B0 08V 01 | P2B0 08V 05 | P2B0 08V 20 |
| Biomax® 10 | P2B0 10V 01 | P2B0 10V 05 | P2B0 10V 20 |
| Biomax® 30 | P2B0 30V 01 | P2B0 30V 05 | P2B0 30V 20 |
| Biomax® 50 | P2B0 50V 01 | P2B0 50V 05 | P2B0 50V 20 |
| Biomax® 100 | P2B 100V 01 | P2B1 00V 05 | P2B1 00V 20 |
| Biomax® 300 | P2B3 00V 01 | P2B3 00V 05 | P2B3 00V 20 |
| Biomax® 500 | P2B5 00V 01 | P2B5 00V 05 | P2B5 00V 20 |
| Biomax® 1000 | P2B0 1MV 01 | P2B0 1MV 05 | P2B0 1MV 20 |
| **Ultracel® PLC Series – Regenerated Cellulose, Composite Construction** | | | |
| 5 kD | P2C0 05V 01 | P2C0 05V 05 | P2C0 05V 20 |
| 10 kD | P2C0 10V 01 | P2C0 10V 05 | P2C0 10V 20 |
| 30 kD | P2C0 30V 01 | P2C0 30V 05 | P2C0 30V 20 |
| 100 kD | P2C1 00V 01 | P2C1 00V 05 | P2C1 00V 20 |
| 300 kD | P2C3 00V 01 | P2C3 00V 05 | P2C3 00V 20 |
| 1000 kD | P2C0 1MV 01 | P2C0 1MV 05 | P2C01MV 20 |
| **Durapore® – Hydrophilic PVDF** | | | |
| 0.1 µm | P2VV PPV 01 | P2VV PPV 05 | P2VV PPV 20 |
| 0.22 µm | P2GV PPV 01 | P2GV PPV 05 | P2GV PPV 20 |
| 0.45 µm | P2HV MPV 01 | P2HV MPV 05 | P2HV MPV 20 |
| 0.65 µm | P2DV PPV 01 | P2DV PPV 05 | P2DV PPV 20 |

# User Guide

## Pod Depth Filters

## Contents



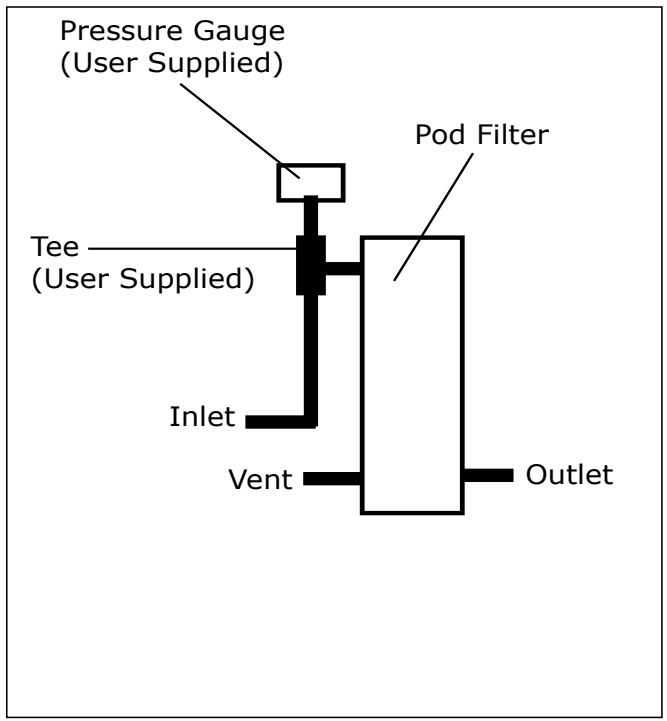

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

# Set-Up

NOTE A tubing kit is available, contact your local representative.

The Process Scale (surface area 0.11, 0.33, 0.55, 0.77, 1.1 and 1.4 $m^2$) Pod filters must be installed in a Pod Holder as described in the Holder User Guide.

1. Connect the inlet port of the filter to the feed line.
2. Connect the outlet port of the filter to the collection line.
3. Connect the vent port of the filter to the vent line.
4. Install a pressure gauge on the inlet or vent line.
5. Attach a vent valve or clamp at the end of the vent tubing.



# Flushing

Flush devices with buffer or purified water prior to use. To fully wet the media, flush the filter as listed in the following tables:

## Clarisolve® Depth Filters

| Media | Flux (LMH) | Lab Scale Pod | | Process Scale Pod | | |
|---|---|---|---|---|---|---|
| | | 0.0135 | 0.027 | 0.11 | 0.33 | 0.55 |
| | | Flow rate (mL/min) | | | | |
| 20MS 40MS 60HX | 600 | 140 | 270 | 1100 | 3300 | 5500 |
| | 300 | 70 | 135 | 550 | 1650 | 2750 |
| | 100 | 23 | 45 | 183 | 550 | 917 |

## Millistak+® Depth Filters

| Media | Flux (LMH) | Lab Scale Pod | | Process Scale Pod | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0.027 | 0.054 | 0.11 | 0.55 | 0.77 | 1.1 | 1.4 |
| | | Flow rate (mL/min) | | | | | | |
| CR | 600 | 270 | 540 | 1100 | 5500 | -- | 11000 | -- |
| HC | 600 | 270 | 540 | 1100 | 5500 | -- | 11000 | -- |
| CE, DE | 600 | 270 | 540 | 1100 | -- | 7700 | -- | 14000 |

## Millistak+® HC Pro Depth Filters

| Media | Flux (LMH) | Lab Scale Pod | | Process Scale Pod | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0.0135 | 0.027 | 0.11 | 0.33 | 0.55 | 0.77 | 1.1 |
| | | Flow rate (mL/min) | | | | | | |
| D0SP | 300 | 70 | 135 | 550 | 1650 | -- | 3850 | -- |
| C0SP | 300 | 70 | 135 | 550 | 1650 | -- | 3850 | -- |
| X0SP | 300 | 70 | 135 | 550 | -- | 2750 | -- | 5500 |

## Flushing Procedure

1. Install new tubing on the inlet, outlet and vent lines of the filter.
2. Attach a pressure gauge to the vent or inlet port to monitor inlet pressure.
3. Attach a vent valve or clamp at the end of the vent tubing.
4. Start flushing the filter at the flow rate listed in the tables.
5. Open the vent to purge any air from the filter. Air purge may be assisted by temporarily clamping the outlet line closed.
6. Close the vent and open the outlet line to allow flow through the filter. For 100 and 300 LMH flow rates, back pressure is recommended to wet the device. To create the back pressure, partially close the outlet valve and completely close the vent valve. Increase the pressure in the device up to 10 psig by partially closing the outlet valve slowly, then vent slowly by opening the outlet to purge any air.
7. Over about 30 seconds, gradually increase the flow to achieve the desired flux rate.
8. Start the flush.
9. Flush until the desired target volume or the desired TOC level is reached (see Conductivity and TOC Test Results).
10. For optimal performance with a Pod filter, run the filtration process with product at a flux of 100 to 300 LMH and a max differential pressure of 2 bar (30 psi).

NOTE It is normal to observe an increase in the hydraulic pressure on the process scale pod holder during operation. **Hydraulic pressure should never exceed 124 bar (1800 psi).**

Optimal flux for CR media is application dependent. Flux rate should be optimized through testing.

11. When filtration is complete, perform a blow down to recover product held up in the device by connecting air supply tubing to the vent port.

| Media | | Blow Down Pressure bar (psi) | Time min |
|---|---|---|---|
| Clarisolve® | | 0.3 (5) | 10 |
| Millistak+® | CR<br>CE<br>DE | 0.3 (5) | 10 |
| | A1HC<br>B1HC<br>C0HC<br>D0HC<br>X0HC<br>F0HC | increase pressure at a rate of 0.1 bar/min (2 psi/min) until 1 bar (15 psi) is reached | up to 5 |
| Millistak+® HC Pro | D0SP<br>C0SP<br>X0SP | increase pressure at a rate of 0.1 bar/min (2 psi/min) until 1 bar (15 psi) is reached | 10 |

# Conductivity and TOC

The information below was generated from a flush with purified water.

## Conductivity and TOC Test Results

Millistak+® HC and Clarisolve ® filters were autoclaved for 60 minutes at 123 °C, then flushed with purified water at a flow rate of 600 LMH to a throughput of 100L/m$^2$. The filter effluent was then tested for conductivity and TOC.

Millistak+ ® HC Pro devices were not autoclaved and were flushed at a flow rate of 300 LMH to a throughput of 50 L/m$^2$.

| Media | Catalog Number (Lot Number) | Flux (LMH) | Test Results | |
|---|---|---|---|---|
| | | | Conductivity (µS/cm) | TOC Value (ppm) |
| **Clarisolve®** | | | | |
| 20MS | CS20MS01L3 (CP2KA10821) | 600 | 1.59 | 1.40 |
| | CS20MS01L3 (CP2KA10822) | 600 | 0.80 | 1.80 |
| | CS20MS01L3 (CP2KA10823) | 600 | 1.29 | 2.10 |
| 40MS | CS40MS01L3 (CP2KA10848) | 600 | 0.91 | 0.90 |
| | CS40MS01L3 (CP2KA10849) | 600 | 1.23 | 1.20 |
| | CS40MS01L3 (CP2KA10850) | 600 | 1.29 | 1.10 |
| 60HX | CS60HX01L3 (CP2KA10842) | 600 | 0.15 | BDL |
| | CS60HX01L3 (CP2KA10843) | 600 | BDL | BDL |
| | CS60HX01L3 (CP2KA10844) | 600 | 0.14 | BDL |
| **Millistak+®** | | | | |
| D0HC | MD0HC01FS1 (CP3NA24863) | 600 | 1.75 | 0.80 |
| | MD0HC01FS1 (CP3NA24864) | 600 | 2.22 | 0.90 |
| | MD0HC01FS1 (CP3NA24864) | 600 | 2.48 | 1.20 |
| C0HC | MC0HC01FS1 (CP3NA24866) | 600 | 1.33 | 0.80 |
| | MC0HC01FS1 (CP3NA24867) | 600 | 1.85 | 0.90 |
| | MC0HC01FS1 (CP3NA24868) | 600 | 2.06 | 1.00 |
| X0HC | MX0HC01FS1 (CP3NA24878) | 600 | 43.87 | 0.90 |
| | MX0HC01FS1 (CP3NA24879) | 600 | 31.44 | 0.80 |
| | MX0HC01FS1 (CP3NA24880) | 600 | 52.26 | 0.90 |
| A1HC | MA1HC01FS1 (CP3NA24860) | 600 | 14.83 | 0.60 |
| | MA1HC01FS1 (CP3NA24861) | 600 | 21.87 | 0.60 |
| | MA1HC01FS1 (CP3NA24861) | 600 | 19.42 | 0.70 |
| F0HC | MF0HC01FS1 (CP9JN75883) | 600 | 40.30 | 1.20 |
| | MF0HC01FS1 (CP9JN75884) | 600 | 21.40 | 0.46 |
| CR40 | MCR4001FS1 (CP8SN71164-13 | 600 | 4.36 | 1.70 |
| | MCR4001FS1 (CP8SN71165-3) | 600 | 4.57 | 1.80 |
| | MCR4001FS1 (CP8SN71166-12) | 600 | 4.56 | 1.60 |
| | MCR4001FS1 (CP8SN71167-11) | 600 | 4.24 | 1.40 |
| | MCR4001FS1 (CP8SN71168-25) | 600 | 4.51 | 1.30 |

| Media | Catalog Number (Lot Number) | Flux (LMH) | Test Results | |
|---|---|---|---|---|
| | | | Conductivity (µS/cm) | TOC Value (ppm) |
| **Millistak+® HC Pro** | | | | |
| D0SP | MD0SP23CL3 (W167597) | 300 | 41.50 | 0.95 |
| | MD0SP23CL3 (W167612) | 300 | 27.50 | 1.09 |
| | MD0SP23CL3 (W167613) | 300 | 24.40 | 0.61 |
| | MD0SP01FS1 (W177017-005) | 300 | 8.98 | 0.47 |
| | MD0SP01FS1 (W177016-011) | 300 | 14.98 | 0.77 |
| | MD0SP01FS1 (W177016-012) | 300 | 16.73 | 0.69 |
| | MD0SP01FS1 (W177016-013) | 300 | 11.12 | 0.70 |
| | MD0SP01FS1 (W177015-005) | 300 | 8.86 | 0.78 |
| C0SP | MC0SP23CL3 (W167602) | 300 | 18.90 | 1.15 |
| | MC0SP23CL3 (W167603) | 300 | 23.40 | 2.01 |
| | MC0SP23CL3 (W167604) | 300 | 37.20 | 3.18 |
| | MC0SP01FS1 (W177025-005) | 300 | 19.20 | 1.24 |
| | MC0SP01FS1 (W177024-011) | 300 | 17.53 | 1.0 |
| | MC0SP01FS1 (W177024-012) | 300 | 14.15 | 0.88 |
| | MC0SP01FS1 (W177024-013) | 300 | 16.37 | 0.74 |
| | MC0SP01FS1 (W177023-005) | 300 | 11.34 | 0.71 |
| X0SP | MX0SP23CL3 (W167607) | 300 | 25.00 | 2.20 |
| | MX0SP23CL3 (W167608) | 300 | 32.80 | 2.90 |
| | MX0SP23CL3 (W167609) | 300 | 24.10 | 3.00 |
| | MX0SP01FS1 (W177033-005) | 300 | 10.35* | 4.9* |
| | MX0SP01FS1 (W177032-014) | 300 | 10.51* | 2.95* |
| | MX0SP01FS1 (W177032-015) | 300 | 11.14* | 2.95* |
| | MX0SP01FS1 (W177032-016) | 300 | 10.64* | 3.39* |
| | MX0SP01FS1 (W177031-005) | 300 | 8.12* | 1.6* |

BDL = Below detection Limit

*TOC and conductivity testing was performed after a 30 minute hold with pure water, followed by a 50 L/m² pure water flush at 300 LMH with 5 psi back pressure for these units.

# Process Optimization

Measure inlet and outlet pressure, original process fluid turbidity, and filtrate turbidity over time to provide data to verify performance and calculate sizing estimates.

# Specifications

## Materials of Construction

| Component | Material | | |
|---|---|---|---|
| **Millistak+® Depth Filters** | | | |
| Housing | Glass filled polypropylene | | |
| Adapters | Glass filled polypropylene | | |
| Gaskets and Plugs | Thermo Elastic Polymer (TPE) | | |
| Filter Media | CR | | Activated Carbon and cellulose fibers |
| | CE | | Cellulose fibers |
| | DE | | Cellulose fibers and diatamaceuous earth |
| | HC | D0HC, C0HC, F0HC, X0HC | Multiple layers of cellulose fibers and diatamaceuous earth |
| | | A1HC, B1HC | Multiple layers of cellulose fibers and diatamaceuous earth; 0.1 µm RW membrane |
| **Clarisolve® Depth Filters** | | | |
| Housing | Glass filled polypropylene | | |
| Adapters | Glass Filled Polypropylene | | |
| Gaskets and Plugs | Thermo Plastic Elastomer (TPE) | | |
| Filter Media | 20MS, 40MS | | Polypropylene and cellulose fibers combined with an inorganic filter aid |
| | 60HX | | Polypropylene |
| **Millistak+® HC Pro Filters** | | | |
| Housing | Glass filled Polypropylene | | |
| Adapters | Glass Filled Polypropylene | | |
| Gaskets and Plugs | Thermo Plastic Elastomer (TPE) | | |

| Component | Material | |
|---|---|---|
| Filter Media | D0SP | Nonwoven, Silica filter aid/ Polyacrylic fiber pulp |
| | C0SP | Silica filter aid/Polyacrylic fiber pulp |
| | X0SP | Silica filter aid/Polyacrylic fiber pulp |

## Operating Parameters

| Parameter | | Lab Scale Pod | Process Scale Pod |
|---|---|---|---|
| **Millistak+® Filters** | | | |
| Effective Surface Area | HC Media | 0.027 $m^2$, 0.054 $m^2$ | 0.11, 0.55, 1.1 $m^2$ |
| | DE, CE Media | | 0.11, 0.77, 1.4 $m^2$ |
| Inlet, Outlet and Vent Connections | | ¼ in. (6 mm) Hose barb | Flat Seal |
| Operating Temperature Range | | 4 to 37 °C | 4 to 37 °C |
| Sterilization | | 2 cycles of 60 minutes at 123° C | 1 cycle of 60 minutes at 123° C |
| Typical Flush/Process Flux | | 100 to 600 LMH | |
| Maximum Differential Pressure | Forward | 2.1 bar (30 psid) at 4-37 °C | 2.1 bar (30 psid) at 25° C; 15 psid (1.0 bar) at 80° C |
| | Reverse | 2.1 bar (30 psid) at 37 °C | 2.1 bar (30 psid) at 25° C |
| Housing Operating Pressure | | 2.1 bar (30 psid) at 37 °C | 3.5 bar (50 psig) at 25 °C; 15 psig (1.0 bar) at 80° C |

| Parameter | | Lab Scale Pod | Process Scale Pod |
|---|---|---|---|
| **Clarisolve® Depth Filters** | | | |
| Effective Surface Area | | 0.0135, 0.027 $m^2$ | 0.11, 0.33, 0.55 $m^2$ |
| Inlet, Outlet and Vent Connections | | ¼ in. (6 mm) Hose barb | Flat seal |
| Operating Temperature Range | | 4 to 37 °C | 4 to 37 °C |
| Sterilization | | Autoclave for two cycles of 60 minutes at 123 °C | Autoclave for one cycle of 60 minutes at 123 °C |
| Typical Flush/Process Flux | | 100 to 600 LMH | |
| Maximum Differential Pressure | Forward | 2.1 bar (30 psid) at ≤ 37 ºC | 2.1 bar (30 psid) at ≤ 25 ºC<br>1.0 bar (15 psid) at ≤ 80 ºC |
| | Reverse | 2.1 bar (30 psid) at ≤ 37 ºC | 2.1 bar (30 psid) at ≤ 25 ºC |
| Housing Operating Pressure | | 2.1 bar (30 psig) at ≤ 37 ºC | 3.5 bar (50 psid) at ≤ 25 ºC<br>1.0 bar (15 psid) at ≤ 80 ºC |
| **Millistak+® HC Pro Filters** | | | |
| Effective Surface Area | D0SP, C0SP | 0.0135 $m^2$ | 0.11, 0.33, 0.77 $m^2$ |
| | | 0.027 $m^2$ | |
| | X0SP | 0.0135 $m^2$ | 0.11, 0.55, 1.1 $m^2$ |
| | | 0.027 $m^2$ | |
| Inlet, Outlet and Vent Connections | | 1/4 in (6 mm) hose barb | Flat seal |
| Operating Temperature Range | | 4 to 40 °C | 4 to 40 °C |
| Sterilization | | Integrity is maintained after 1 cycle of 60 minutes at 123 °C. Recommended for post-use decontamination only. | |
| Typical Flush/Process Flux | | 100 to 600 LMH | |

| Parameter | | Lab Scale Pod | Process Scale Pod |
|---|---|---|---|
| Maximum Differential Pressure | Forward | 2.1 bar (30 psid) at ≤ 40 ºC | 2.1 bar (30 psid) at ≤ 80 ºC |
| | Reverse | 2.1 bar (30 psid) at ≤ 25 ºC | 2.1 bar (30 psid) at ≤ 25 ºC |
| Housing Operating Pressure | | 2.1 bar (30 psid) at 40 °C | 3.5 bar (50 psid) at 80 ºC |

Pressure must be monitored at inlet or vent connections.

# Typical Hold-up and Void Volumes

## Clarisolve® Depth Filters

| Parameter | Hold-up volume (L) | | | | |
|---|---|---|---|---|---|
| | Lab Scale Pod | | Process Scale Pod | | |
| | 0.0135 | 0.027 | 0.11 | 0.33 | 0.55 |
| **20MS Media** | | | | | |
| after gravity drain | 0.26-0.32 | 0.51-0.57 | 3.1-3.4 | 8.4-9.0 | 13.5-14.5 |
| after blow down | 0.13-0.19 | 0.30-0.35 | 0.50-0.70 | 1.8-2.4 | 3.1-4.1 |
| **40MS Media** | | | | | |
| after gravity drain | 0.26-0.32 | 0.51-0.57 | 3.1-3.4 | 8.4-9.0 | 13.5-14.5 |
| after blow down | 0.13-0.19 | 0.30-0.35 | 0.50-0.70 | 1.8-2.4 | 3.1-4.1 |
| **60HX Media** | | | | | |
| after gravity drain | 0.30-0.36 | 0.53-0.59 | 3.2-3.5 | 7.7-8.3 | 12.8-13.8 |
| after blow down | 0.10-0.16 | 0.24-0.29 | 0.30-0.50 | 3.3-3.6 | 3.0-3.4 |

## Millistak+® HC Pro Depth Filters

| Parameter | | Hold-up volume (L) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Lab Scale Pod | | Process scale pod | | | | |
| | | 0.0135 | 0.027 | 0.11 | 0.33 | 0.55 | 0.77 | 1.1 |
| **D0SP Media** | | | | | | | | |
| Internal void volume (L) | | 0.275 | 0.541 | 2.12 | 5.50 | -- | 12.9 | -- |
| Hold-up volume (L) | after gravity drain | 0.198 | 0.397 | 1.00 | 2.50 | -- | 6.30 | -- |
| | after blow down | 0.094 | 0.139 | 0.643 | 1.99 | -- | 5.04 | -- |
| **C0SP Media** | | | | | | | | |
| Internal void volume (L) | | 0.259 | 0.618 | 2.04 | 5.81 | -- | 12.8 | -- |
| Hold-up volume (L) | after gravity drain | 0.188 | 0.506 | 1.13 | 2.81 | -- | 6.62 | -- |
| | after blow down | 0.119 | 0.185 | 0.711 | 2.22 | -- | 5.44 | -- |
| **X0SP Media** | | | | | | | | |
| Internal void volume (L) | | 0.347 | 0.676 | 1.25 | -- | 4.94 | -- | 9.47 |
| Hold-up volume (L) | after gravity drain | 0.188 | 0.395 | 0.801 | -- | 3.32 | -- | 6.72 |
| | after blow down | 0.09 | 0.175 | 0.635 | -- | 2.92 | -- | 5.85 |

# **Chromaflow** columns

PROCESS COLUMNS

Chromaflow™ columns are a family of convenient to use, process-scale columns. A patented nozzle in the top and bottom of the column allows packing, unpacking, and cleaning when fully assembled, that is with the lid in place. Chromaflow columns simplify chromatographic procedures and offer:

- convenience
- saving of labor
- reproducibility
- contained packing
- scalability

## General column description

Chromaflow low-pressure columns (Fig 1) are available in a choice of dimensions and materials. The complete range offers inner diameters (i.d.) from 300 to 2000 mm (Table 1), with column tubes manufactured from cast acrylic (Fig 1). All dimensions are available with variable bed heights, providing a wide variety of bed volumes. All columns are pressure rated for operation at 3 bar.

Chromaflow columns incorporate a patented, pack-in-place nozzle (Fig 2) through which process liquids enter and exit. Manual or automated versions of the nozzles are available. The automated nozzle is controlled from the packing station or the nozzle control unit. The nozzle has three positions to facilitate the different aspects of column operation: packing, operation, unpacking and cleaning. In addition to this pack-in-place functionality, the nozzle also contains the process liquid flow path to provide a consolidated solution to the process stream handling.

Bed supports are available in 316L stainless steel or polyethylene. The multilayer, woven stainless steel bed supports have very high chemical resistance and longevity for use in applications where salt concentrations are low and pH is above 5. Polyethylene bed supports are recommended for applications with low pH and high salt concentrations. All other wetted parts in columns with polyethylene bed supports are manufactured from plastic or noncorrodible materials for use in low pH /high salt applications.


**Fig 1.** A Chromaflow column, 2000 mm i.d.

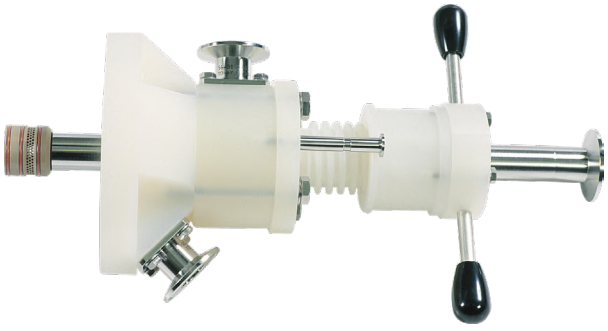


**Fig 2.** The Chromaflow nozzle that enables packing in place in a fully assembled column (Cytiva patent).

The construction materials include 316L stainless steel, acrylic, polypropylene, polyethylene, PEEK 450 G, EPDM rubber and FEP encapsulated silicone. These materials have high chemical resistance to the liquids typically used in process chromatography (Table 2). Furthermore, all polymeric materials are approved according to USP class VI tests for toxicity.

As an option, a dedicated packing station is available for Chromaflow columns. The packing station speeds up the packing procedure by eliminating the more time-consuming, manual maneuvers (Fig 3).

Comprehensive documentation is delivered with each column and includes a User manual, a Maintenance manual, assembly drawings, a full spare part list, materials certificates, etc.

A Validation Support File containing information on column component composition, materials of construction and toxicity studies is also available.

**Fig 3.** Packing Chromaflow columns with the dedicated packing station is convenient and simple.

## Convenient and labor saving

Once the column is assembled and the lid in place, no lifting gear is required for packing, operation, unpacking or cleaning-in-place (CIP). This means that a single operator can perform all column operations, thereby reducing labor costs and increasing convenience in large-scale operations.

## Reproducibility

Packing with the lid in place allows the packing parameters to be easily set and fixed. Manual operation is minimized and standard operating procedures can be followed, helping to give reproducible column packing and results.

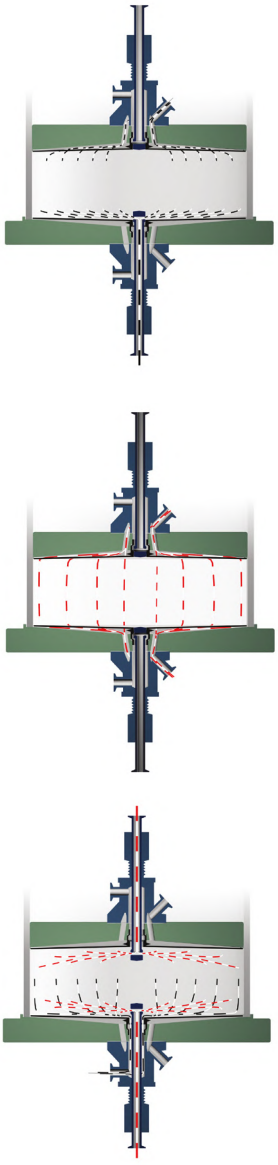
## Contained packing

Improved safety is another advantage of the Chromaflow column concept. Because all the column operations are performed in a "closed system" environment, there is less risk of the operator coming into contact with hazardous chemicals and of the target product being exposed to contamination. In this way, overall safety and hygienic operation are improved.

## Principle of operation

The column has a three-position nozzle located in the center of the top and bottom bed support. These three positions enable packing, unpacking, operation and cleaning to be performed without any adjustments to the assembled column, that is the lid remains in place.

Flow profiles from the two nozzles are identical. Packing direction will depend on the characteristics of the media and packing method used. The three positions are illustrated in Figure 4.



**Packing position**
The bottom nozzle is extended part of the way (mid position) into the column. The top nozzle is fully retracted. Slurry enters the column via the bottom nozzle and excess liquid exits via the top mobile phase outlet. After packing, the slurry lines are isolated from the mobile phase and can be cleaned independently from the rest of the column.

**Running position**
The bottom and top nozzles are retracted. Mobile phase enters the column directly into an annulus, immediately behind the bed support. The annulus is cut through at an angle to ensure that linear flow is kept constant during distribution of the mobile phase across the bed.

**Unpacking position**
In this position, both bottom and top nozzles are fully extended into the column thereby exposing a third passage through which medium leaves the column.

Cleaning solution can be pumped through the nozzles and sprayed into the column. In this way the column is easily and effectively cleaned without exposing the interior or the medium to the environment, and without dismantling the column.

**Fig 4.** The three positions of the Chromaflow nozzle showing packing from the top.

## Scalability

Chromaflow columns are available in a wide range of dimensions, all designed and constructed around the same design principle. Standard range columns come in dimensions from 400 to 1000 mm. For more information about columns and dimensions, see Ordering information. Scaling up a chromatographic process from small to larger diameters is easily performed with maintained reproducibility, safety and convenience.

## Column dimensions

A selection of Chromaflow columns in the range 400 to 2000 mm i.d. are presented in Table 1. The adapter stroke length is a standard 200 mm. Variable bed heights are available in the ranges 100 to 300 mm, 200 to 400 mm and 300 to 500 mm.

## Chromaflow 400 SFP columns

Chromaflow 400 SFP (small flow path) columns are specially designed for low-flow applications. The dimensions in the mobile phase have been optimized to reduce dead volumes to a minimum and the area behind the nozzle tip has also been reduced.

## Column materials and their chemical resistance

Table 2 lists the major components of Chromaflow columns in contact with process fluids (wetted parts) and Table 3 lists the chemical resistance of materials using data compiled from several published sources. It is important to note that columns with stainless steel bed supports and other stainless steel wetted components must be appropriately maintained when exposed to NaCl. Since salt can be corrosive to stainless steel over time, it is recommended that residual salt is removed by rinsing columns with at least five column volumes (CV) of clean water.

**Table 1.** Weights, volumes and dimensions for variable bed height Chromaflow columns

| Description | Max operating pressure (bar) | Volume (L) | Column overall height (mm) | Weight, dry (kg) | Footprint (mm × mm) |
|---|---|---|---|---|---|
| Chromaflow column 400/100-300* | 3 | 12.6–37.8 | 1568 | 230 | 700 × 700 |
| Chromaflow column 600/100-300 | 3 | 28.3–84.9 | 1568 | 375 | 800 × 800 |
| Chromaflow column 800/100-300 | 3 | 50.3–150.9 | 1572 | 610 | 1000 × 1000 |
| Chromaflow column 1000/100-300 | 3 | 78.5–235.5 | 1573 | 930 | 1200 × 1200 |
| Chromaflow column 1200/100-300 | 3 | 113.1–339.3 | | | |
| Chromaflow column 1400/100-300 | 3 | 153.9–461.7 | | | |
| Chromaflow column 1600/100-300 | 3 | 201.1–603.3 | | | |
| Chromaflow column 1800/100-300 | 3 | 254.5–763.5 | | | |
| Chromaflow column 2000/100-300 | 3 | 314.2–942.6 | | | |

* The first figure in the column name indicates the inner diameter and the second figure indicates stroke length.

**Table 2.** Major components and their composition

| Component | Material | In contact with process stream |
|---|---|---|
| Column tube | Acrylic or stainless steel 316L | Yes |
| Column lids | Stainless steel 316L | No |
| Distributor | Polypropylene | Yes |
| Bed support | Stainless steel 316L or polyethylene | Yes |
| Chromaflow nozzle | Polypropylene, stainless steel 316L, PEEK 450 G | Yes |
| Seals | EPDM or FEP encapsulated silicone | Yes |
| Stand | Stainless steel 316 | No |

EPDM = ethylene propylene diene, FEP = fluoroethenepropene, PEEK = polyetherether ketone

**Table 3.** Chemical resistance of materials used in Chromaflow columns (60 days)

| Chemical | Acrylic | SS 316L | EPDM | FEP | PEEK 450 G | PE | PP |
|---|---|---|---|---|---|---|---|
| Acetic acid 1.7 M | + | + | + | + | + | + | + |
| EtOH 20%[1] | + | + | + | + | + | + | + |
| EtOH 40% | – | + | + | + | + | (+) | + |
| Ethylene glycol 50% | + | + | + | + | + | + | + |
| Formaldehyde 1.7 M | + | + | + | + | + | + | + |
| Formic acid 10% | (+) | + | + | + | + | + | + |
| Glycerol 100% | + | + | + | + | + | + | + |
| Hydrochloric acid 0.1 M | + | – | + | + | + | + | + |
| Isopropyl alcohol 30% | – | + | + | + | + | (+) | + |
| Nitric acid 0.1 M | + | + | + | + | + | (+) | + |
| Phosphoric acid 25% | + | (+) | + | + | + | + | + |
| Sodium chloride 0.5 M | + | +[2] | + | + | + | + | + |
| Sodium hydroxide 2 M[3] | + | + | + | + | + | + | + |
| Trifluoroacetic acid 0.1% | (+) | + | + | + | + | + | + |
| Triton™ X-100 100% | + | + | + | + | + | + | + |
| Urea 8 M | + | + | + | + | + | + | + |

+ Resistant (+) Limited resistance – Not recommended

[1] Do not expose acrylic to concentrations of ethanol greater than 20%. Do not exceed the following parameters during storage: 5 yr, 23°C, 0.5 bar g.
[2] NaCl can cause corrosion on stainless steel at pH <5. Do not use NaCl in storage solutions. Rinse with at least 5 CV of clean water after use with NaCl.
[3] Maximum exposure 4 h.

SS=stainless steel, EPDM=ethylene propylene diene, FEP=fluoroethenepropene, PEEK=polyetherether ketone, PE=polyethylene, PP=polypropylene.

## Sanitizing Chromaflow columns

The design of Chromaflow columns facilitates cleaning-in-place. Below is a recommended cleaning protocol suitable for most applications.

1. Circulate 1.5 CV of 20% acetic acid at a low flow velocity (60 cm/h) for 15 min, upward flow. Then reverse the flow for 15 min.
2. Repeat this procedure with 1.0 M NaOH.
3. Following step 2, slowly circulate 1.0 M NaOH in the column for 60 min.
4. Re-equilibrate the column with a storing or starting buffer.

## Chromaflow Packing stations

Chromaflow Packing stations make column priming and packing a simple operation, reducing the operator's time to a minimum. The packing stations consist of a control panel with pumps and valves fitted underneath (Fig 5). Valves and diaphragm pumps are actuated pneumatically from the control panel. As they are brought into operation indicators on the control panel display the relevant flow paths. For operation, packing stations only require a supply of compressed air. To select an appropriate packing station for your column and media, refer to Tables 4 and 5.


**Fig 5.** Chromaflow Packing station Pack 100.

**Table 4.** Specifications of Chromaflow packing stations

| Designation* | Pump | Pump flow capacity (L/min) | Req. air supply ($m^3$/min) | Inlet piping/ outlet i.d. (mm) | TC connections (mm) | Weight, dry (kg) | Size W × H × D (mm) |
|---|---|---|---|---|---|---|---|
| Pack 50 | Tapflo™ T53 | 10–50 | 0.5 | 22.1/22.1 | 50.5 | 115 | 810 × 1175 × 715 |
| Pack 100 | Tapflo T103 | 30–100 | 1.0 | 34.8/22.1 | 50.5 | 130 | 810 × 1175 × 715 |

* Packing stations, Pack 200 and Pack 400 with pump flow capacities of 60 to 200 l/min and 100 to 400 l/min are available as custom orders.

**Table 5.** Approximate packing flow rates for different media at two different bed heights

| Column diameter (mm) | 400 | | | | 600 | | | | 800 | | | | 1000 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bed height (mm)** | **150** | | **300** | | **150** | | **300** | | **150** | | **300** | | **150** | | **300** | |
| **Flow** | **cm/h** | **L/min** | **cm/h** | **L/min** | **cm/h** | **L/min** | **cm/h** | **L/min** | **cm/h** | **L/min** | **cm/h** | **L/min** | **cm/h** | **L/min** | **cm/h** | **L/min** |
| Sepharose™ Fast Flow media | 500 | 11 | 250 | 5.5 | 500 | 24 | 250 | 12 | 500 | 42 | 250 | 21 | 500 | 66 | 250 | 33 |
| Sepharose Big Beads media | 1600 | 34 | 1200 | 25 | 1600 | 75 | 1200 | 57 | 1600 | 134 | 1200 | 101 | 1600 | 209 | 1200 | 157 |

# What else do I need?

### *The column*

The columns are supplied ready for use and are equipped with adjustable feet. Castors can be ordered separately for columns up to 1000 mm in diameter.

### *Isolating the column after packing*

We recommend using sanitary stainless steel valves (of the appropriate inner diameter) on the mobile phase to prevent contamination of the packed bed. For storage purposes, blind flanges with a clamp and gasket can be used to seal off the column.

### *Connecting the column to your system and packing station*

Clamps and gaskets of suitable size are required to connect the sanitary flanged inlet/outlet to either valves or tubing of the same type. Preflanged tubing is also available.

### *Assembly or disassembly of the column*

An adequate sized wrench is needed for assembly or disassembly of the column. A hoist is needed to remove the adapter or top lid from the column.

### *Spare parts to keep on site*

It is recommended that nozzle seals, column seals, and column bed support kits are kept as spare parts.

### *Useful accessories*

**Safety valve:** Precalibrated valve which releases pressure if the calibrated value is exceeded. Recommended to install on the mobile phase inlet if no other pressure sensor is included in the chromatography system. The T-junction, clamps and gaskets must be ordered separately.

**Pressure sensor:** The sensor is installed inline, preferably on the mobile phase inlet. Clamps and gaskets have to be ordered separately.

# Ordering information

## *Columns*

| **Chromaflow columns with acrylic tubes** | **Bed support 10 mm SS sinter** | **Bed support 20 mm SS sinter** | **Bed support 20 mm PE sinter** |
|---|---|---|---|
| *I.d. 400 mm Man. nozzle* | | | |
| Stroke length 100-300 | 18-1150-40 | 18-1159-40 | 18-1161-40 |
| Stroke length 200-400 | 18-1157-42 | 18-1159-42 | 18-1161-42 |
| Stroke length 300-500 | 18-1157-44 | 18-1159-44 | 18-1161-44 |
| *I.d. 400 mm Auto. nozzle* | | | |
| Stroke length 100-300 | 18-1157-41 | 18-1159-41 | 18-1161-41 |
| Stroke length 200-400 | 18-1157-43 | 18-1159-43 | 18-1161-43 |
| Stroke length 300-500 | 18-1157-45 | 18-1159-45 | 18-1161-45 |
| *I.d. 400 mm SFP* Man. nozzle* | | | |
| Stroke length 100-300 | 18-1170-53 | 18-1176-12 | 11-0011-85 |
| Stroke length 200-400 | 11-0011-80 | 11-0011-83 | 11-0011-86 |
| Stroke length 300-500 | 11-0011-82 | 11-0011-84 | 11-0011-87 |
| *I.d. 400 mm SFP Auto. nozzle* | | | |
| Stroke length 100-300 | 11-0011-89 | 11-0011-91 | 11-0011-94 |
| Stroke length 200-400 | 11-0011-88 | 11-0011-92 | 11-0011-95 |
| Stroke length 300-500 | 11-0011-90 | 11-0011-93 | 11-0011-96 |
| *I.d. 600 mm Man. nozzle* | | | |
| Stroke length 100-300 | 18-1150-60 | 18-1159-60 | 18-1161-60 |
| Stroke length 200-400 | 18-1157-62 | 18-1159-62 | 18-1161-62 |
| Stroke length 300-500 | 18-1157-64 | 18-1159-64 | 18-1161-64 |
| *I.d. 600 mm Auto. nozzle* | | | |
| Stroke length 100-300 | 18-1157-61 | 18-1159-61 | 18-1161-61 |
| Stroke length 200-400 | 18-1157-63 | 18-1159-63 | 18-1161-63 |
| Stroke length 300-500 | 18-1157-65 | 18-1159-65 | 18-1161-65 |
| *I.d. 800 mm Man. nozzle* | | | |
| Stroke length 100-300 | 18-1150-80 | 18-1159-80 | 18-1161-80 |
| Stroke length 200-400 | 18-1157-82 | 18-1159-82 | 18-1161-82 |
| Stroke length 300-500 | 18-1157-84 | 18-1159-84 | 18-1161-84 |
| *I.d. 800 mm Auto. nozzle* | | | |
| Stroke length 100-300 | 18-1157-81 | 18-1159-81 | 18-1161-81 |
| Stroke length 200-400 | 18-1157-83 | 18-1159-83 | 18-1161-83 |
| Stroke length 300-500 | 18-1157-85 | 18-1159-85 | 18-1161-85 |
| *I.d. 1000 mm Man. nozzle* | | | |
| Stroke length 100-300 | 18-1150-10 | 18-1160-10 | 18-1162-10 |
| Stroke length 200-400 | 18-1158-12 | 18-1160-12 | 18-1162-12 |
| Stroke length 300-500 | 18-1158-14 | 18-1160-14 | 18-1162-14 |
| *I.d. 1000 mm Auto. nozzle* | | | |
| Stroke length 100-300 | 18-1158-11 | 18-1160-11 | 18-1162-11 |
| Stroke length 200-400 | 18-1158-13 | 18-1160-13 | 18-1162-13 |
| Stroke length 300-500 | 18-1158-15 | 18-1160-15 | 18-1162-15 |

For column specifications other than listed in the table, please contact your local Cytiva representative.
* SFP = Small Flow Path on mobile phase, only available on 400 mm i.d. columns.

### *Packing stations*

| | Min (L/min) | Max (L/min) | Code number |
|---|---|---|---|
| Pack 50 | 10 | 50 | 18-1163-74 |
| Pack 100 | 30 | 100 | 18-1162-08 |
| Pack 200 | 60 | 200 | Custom order |
| Pack 400 | 100 | 400 | Custom order |

# Accessories

| Designation | Code number |
|---|---|
| *Valves* | |
| 4 port 2 way, i.d. 10 mm, 25 mm TC | 18-1012-56 |
| 4 port 4 way, i.d. 10 mm, 25 mm TC | 18-1012-57 |
| 3 port 2 way, i.d. 15 mm, 25 mm TC | 44-5499-90 |
| 4 port 4 way, i.d. 20 mm, 51 mm TC | 44-2302-01 |
| 3 port 2 way, i.d. 22 mm, 51 mm TC | 44-1583-01 |
| 3 port 2 way, i.d. 35 mm, 51 mm TC | 44-5494-65 |
| Valve sealing washer<br>Fits 10 mm 2- and 4-way valves | 18-1128-69 |
| *PVC tubing with sanitary fitting 25 mm TC* | |
| i.d. 10 mm, 900 mm | 18-1012-62 |
| i.d. 10 mm, 1400 mm | 18-1012-63 |
| i.d. 10 mm, 1700 mm | 18-1012-64 |
| i.d. 10 mm, 2000 mm | 18-1012-87 |
| i.d. 14 mm, 750 mm | 18-1027-28 |
| i.d. 14 mm, 1800 mm | 18-1027-29 |
| *PVC tubing with sanitary fitting 51 mm TC* | |
| i.d. 22 mm, 900 mm | 44-1616-09 |
| i.d. 22 mm, 1400 mm | 44-1616-08 |
| i.d. 22 mm, 2000 mm | 44-1616-07 |
| i.d. 22 mm, 4000 mm | 44-1616-06 |
| *Clamp gasket* | |
| 25 mm i.d., 10 mm | 18-1035-79 |
| 25 mm i.d., 12 mm | 18-0200-00 |
| 51 mm i.d., 22 mm | 44-7133-01 |
| 51 mm i.d., 38 mm | 44-0515-01 |
| Clamp 25 mm | 18-1001-31 |
| Clamp 51 mm | 44-7134-01 |
| Blind flange 25 mm incl. gasket | 18-1001-25 |
| Blind flange 51 mm incl. gasket | 44-7135-01 |
| Safety valve, 3 bar, 51 mm TC | 18-5738-01 |
| Safety valve, 5 bar, 51 mm TC | 44-5498-97 |
| T-junction i.d., 10 mm, 2×25 mm TC, 1×51 mm TC | 18-1003-63 |
| Castors, assembly kit 400-600 | 18-1171-51 |
| Castors, assembly kit 800-1000<br>The kit contains a complete set of wheels, fasteners and adapters for a column. | 18-1171-52 |
| Pressure sensor i.d. 10 mm, 25 mm TC | 44-0507-02 |
| Pressure sensor i.d. 22 mm, 51 mm TC | 44-0507-03 |

| Designation | Code number |
|---|---|
| *Media stirrers* | |
| Media stirrer, 80 cm | 18-1149-80 |
| Media stirrer, 150 cm | 18-1149-81 |
| *Connectors* | |
| i.d. 10, 25 mm TC-3/4"-20 UNF threaded | 18-1012-68 |
| i.d. 10, 25 mm TC-i.d. 14, 51 mm TC | 18-1027-25 |
| i.d. 14, 51 mm TC-i.d. 22, 51 mm TC | 18-1027-26 |
| Chromaflow Nozzle control unit | 18-1164-61 |
| *Chromaflow Nozzle pipings* | |
| Chromaflow Nozzle piping 400 1/2" | 18-1172-01 |
| Chromaflow Nozzle piping 400 3/4" | 18-1172-00 |
| Chromaflow Nozzle piping 400 1" | 18-1171-99 |
| Chromaflow Nozzle piping 600 1/2" | 18-1172-06 |
| Chromaflow Nozzle piping 600 3/4" | 18-1172-05 |
| Chromaflow Nozzle piping 600 1" | 18-1172-04 |
| Chromaflow Nozzle piping 800 1/2" | 18-1171-94 |
| Chromaflow Nozzle piping 800 3/4" | 18-1171-93 |
| Chromaflow Nozzle piping 800 1" | 18-1171-92 |
| Chromaflow Nozzle piping 1000 1/2" | 18-1172-09 |
| Chromaflow Nozzle piping 1000 3/4" | 18-1172-08 |
| Chromaflow Nozzle piping 1000 1" | 18-1172-07 |

## cytiva.com/bioprocess



CY13377-17Jul20-DF

cytiva

# Chromaflow™ 400-1000 columns

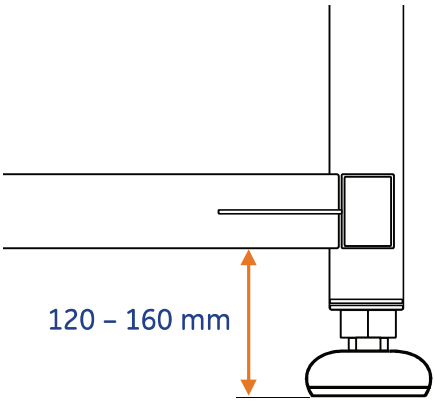
## Operating Instructions

### Original instructions

**NOTICE**

Make sure that the column is level after it has been moved. If the column is not level, column performance may be negatively affected.

| Step | Action |
|---|---|
| 1 | Place two spirit levels diagonally across the top of the column flange. |
| 2 | Place jacks or similar suitable lifting equipment under the column stand. |
| 3 | Use the jacks or the lifting equipment, to carefully relieve the pressure on the column feet. Make sure that uneven load is not placed on the feet.<br>***Note:***<br>*Do not lift the column more than is absolutely necessary to avoid risk of tipping.* |
| 4 | Move the spirit level to different places around the column flange, changing the angle measured by 90 degrees each time, to make sure that the column is level in all directions. |
| 5 | Loosen the locking nuts on the column feet. |
| 6 | Adjust the level of the column by screwing the column feet away from the column stand as required until the feet touch the floor and the column stands level on the floor. Measure to make sure that the feet are extended between 120 to 160 mm from the floor and the base of the stand. |




| | |
|---|---|
| 7 | Move the spirit level around the column flange as described in step 4 to make sure that the column is level in all directions. |

# HyPerforma Single-Use Mixer with Touchscreen Console

### The next generation of efficiency and performance

The Thermo Scientific™ HyPerforma™ Single-Use Mixer (S.U.M.) with Touchscreen Console offers enhanced functionality, ease of use, and efficiency. The complete HyPerforma S.U.M. system consists of a mixer tank, available in 50, 100, 200, 500, 1,000, and 2,000 L sizes with the Touchscreen Console. The HyPerforma S.U.M. has a 5:1 turndown mixing ratio and maintains traditional stirred-tank mixer design principles with a directly coupled motor impeller drive assembly and a cylindrical tank with a specific height-to-diameter ratio. This allows quick turnaround times for both liquid-to-liquid mixing and powder-to-liquid mixing.

### 2,000 L HyPerforma S.U.M. standard configurations

- AC motor
- 3x load cell weighing system
- Touchscreen Console

### Critical upstream application steps

- Media preparation
- Final formulation steps
- Buffer preparation
- Large-volume mixing

### Critical downstream application steps

- Pooling and liquid transfer
- Product suspension
- Mixing and storing multiple batches
- Buffer preparation
- Viral inactivation

### Touchscreen Console capabilities

The Touchscreen Console offers state-of-the-art in-process monitoring and automation capability for the HyPerforma S.U.M. Its modular design allows for an easy-to-use custom user interface. Capabilities include: control of agitation speed, pumps, pinch clamps, and temperature control unit. Users can easily view measurements from load cells, pH sensors, conductivity sensors, resistance temperature detectors (RTDs), and pressure sensors.

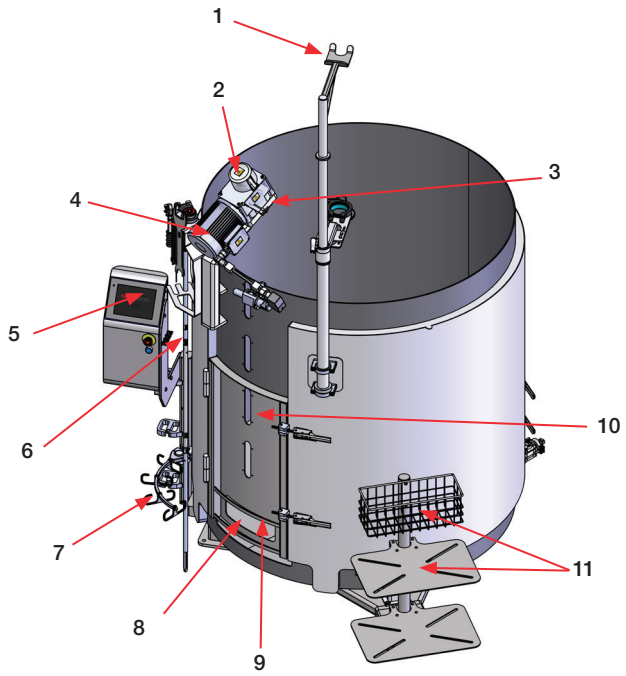
Simple, routine processes can be automated by utilizing measurement values to control the pumps, temperature control unit (TCU), and agitation motor. The Touchscreen Console can help users semi-automate their formulation, pH, or saline titrations, and viral inactivation processes. This allows users to program their HyPerforma S.U.M. for a process and trust that the measurements are accurate, precise, and controlled. The data measured during a process can be exported remotely via Ethernet, Profibus, or Modbus remote terminal unit (RTU), and can also be accessed locally with a USB flash drive.

ThermoFisher SCIENTIFIC

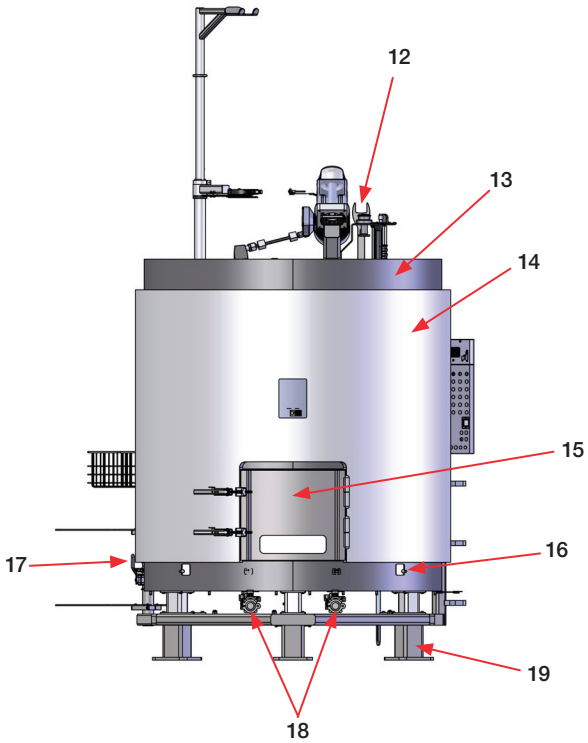
## HyPerforma S.U.M. design features and options

1. Powder hanger for 1 kg, 5 kg, and 25 kg Thermo Scientific™ Powdertainer™ BioProcess Containers (BPCs)
2. Mixing assembly with shield
3. Bearing port receiver with clamp
4. Mixer motor
5. Touchscreen Console
6. Drive shaft, stored
7. Cable management hooks
8. Probe access windows
9. Probe clip hangers
10. Liquid sight window
11. Shelves and basket (optional)
12. Standard tool set: 10 mm (3/8 in.) x 16.9 Nm (150 in.-lb.)square torque wrench, load cell and motor cap lockout wrench
13. 0.95 cm (3/8 in.) dimpled jacket
14. Stainless steel outer support container
15. Rear door (for BPC loading), with sight window
16. Bottom cutouts/pins for BPC attachment and alignment
17. Bleed valve (jacketed models only)
18. 3.81 cm (1.5 in.) tri-clamp connection ports for water inlet/outlet (jacketed models only)
19. Leveling feet (3)

**Front view**

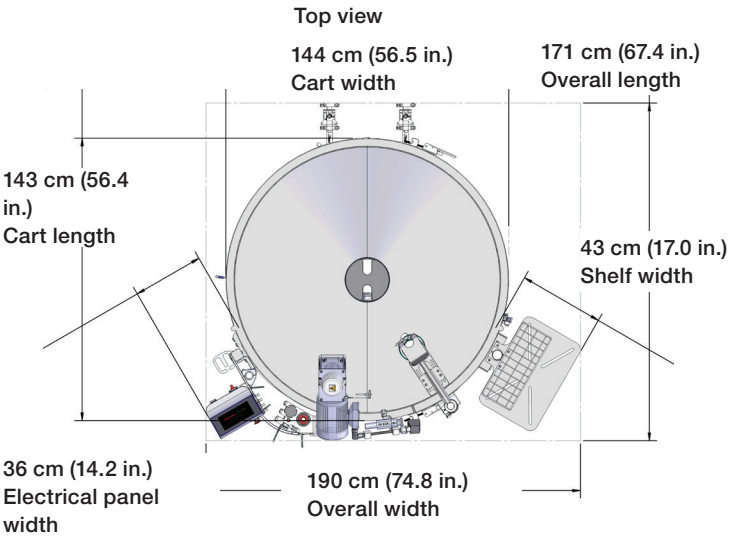


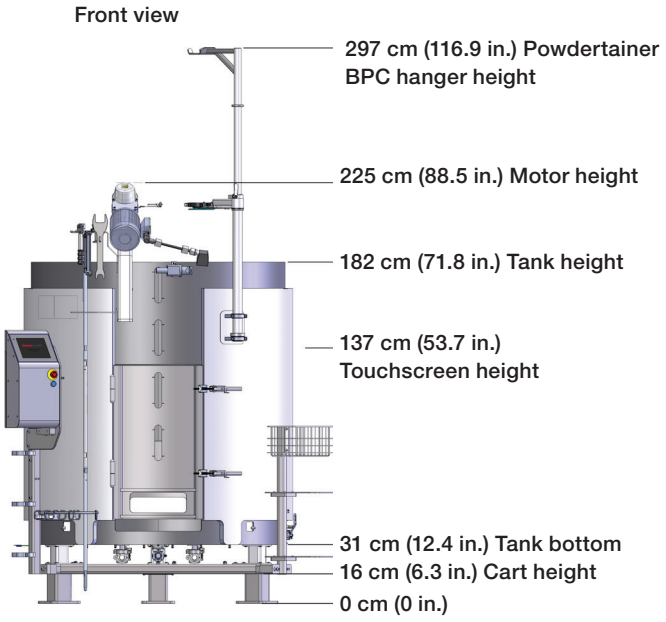

**Back view**




Note: Models without water jackets include the same features as the water-jacketed models shown here, but without the jacket and inlet/outlet ports.
Optional load cells and cable management system are not shown. See the accessories section for more information about these items.

## HyPerforma S.U.M. design specifications




| Touchscreen Console measurement options and specifications | | | | | |
|---|---|---|---|---|---|
| | Load cell | Temperature | pH | Conductivity | BPC and in-line liquid pressure |
| Accuracy after calibration | ±0.5% of full scale | 0.2°C | ±0.05 pH unit | ±5% | ±3.5% of full scale (30 psi) |
| Calibration | 1 to 3 points and zero/tare function | 1 to 3 points | 1 to 3 points | 1 to 3 points | 1 point; zero/tare function |
| Resolution | 0.1 kg | 0.01°C | 0.01 pH unit | 1 µS/cm | 0.01 psi |
| Sensor range | 0 to 3,300 kg | 0 to 200°C | 0 to 14 | 20 to 20,000 µS/cm | 0 to 30 psi |
| Measurement units | kg | °C | pH units | µS/cm | psi |
| Probe type | 3 x Mettler Toledo 0745A load cells | RTD | Electrochemical with 225 mm S8 connector | Two-pole conductivity sensor | Single-use sensor part of the BPC and/or fluid transfer assembly design |

## Accessories

### Sensors and pinch valves

Reusable pH and conductivity probes as well as single-use pressure sensors have been approved and qualified for use with the Touchscreen Console. pH and conductivity measurements can be used to control titration pumps, which enable automatic titration capabilities. The pressure sensors are used in the BPC or line sets to monitor the BPC or liquid pressure, respectively. The BPC can be filled with the proper amount of air when using the pressure sensor in the BPC. The liquid pressure module in the Touchscreen Console is used to control a transfer pump, based on the liquid pressure. Optional pneumatic pinch valves can be used on the fill and harvest line. These valves automatically open and shut when using the fill and/or harvest modules in the Touchscreen Console.

### Ordering information

| Standards | Manufacturer | Cat. No. |
|---|---|---|
| pH sensor | Thermo Fisher Scientific | SV51147.02 |
| | Mettler Toledo | SV51147.01 |
| | Broadley James | SV51147.03 |
| Conductivity sensor | JUMO | SV51148.01 |
| | Mettler Toledo | SV51148.02 |
| Pressure sensor (single use, included in BPC and/or fluid transfer assembly) | PendoTECH 3/8 in. ID tubing | SV20826.05 |
| | PendoTECH 1/2 in. ID tubing | SV20826.01 |
| Pinch valve, harvest line | Thermo Fisher Scientific | SV51108.08 |
| Pinch valve, fill line | Thermo Fisher Scientific | SV51108.05 |

### Heavy-duty tubing clamps

Heavy-duty clamps are used to pinch off line sets that are not in use, to prevent process fluids from escaping. Prior to sterile probe insertion, tubing clamps must be in place to close off probe ports.

### Ordering information

| Description | Cat. No. |
|---|---|
| Heavy-duty tubing clamp (single) | SV20664.01 |
| Heavy-duty tubing clamp (10 pack) | SV20664.04 |

### Load cells

Load cells are typically radially mounted in sets of three. The mounting location varies slightly for each size in order to allow easy access to the bottom drain or sparging mechanisms and tubing.

### Probe clips

Probe clips are used to hold the probes in place on the S.U.M. tank. The independently movable probe clips hang on a thin brace above the probe port tank cutout.

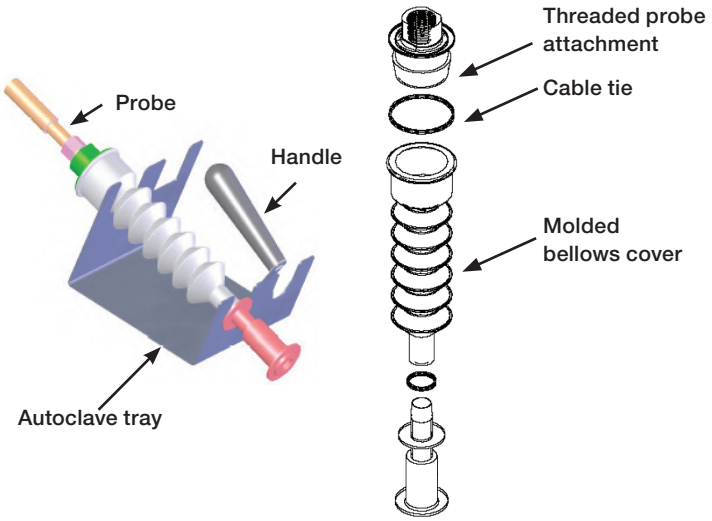
### Ordering information

| Description | Cat. No. |
|---|---|
| 4 plastic probe clips | SV50177P.01 |

### Autoclave tray and probe assembly

The autoclave tray holds the electrochemical probes and bellows in place during the autoclave sterilization process. Design elements include the following:

- Fabricated from stainless steel
- Plastic handle provides for easy transport right out of the autoclave
- Positions probes on 15% incline for greater probe and membrane longevity
- Prevents probe bellows from collapsing during sterilization
- Probe holder accommodates two probes




### Ordering information

| Description | Cat. No. |
|---|---|
| Autoclave tray (stainless steel with plastic carrying handle) | SV50177.01 |

### Cable management system

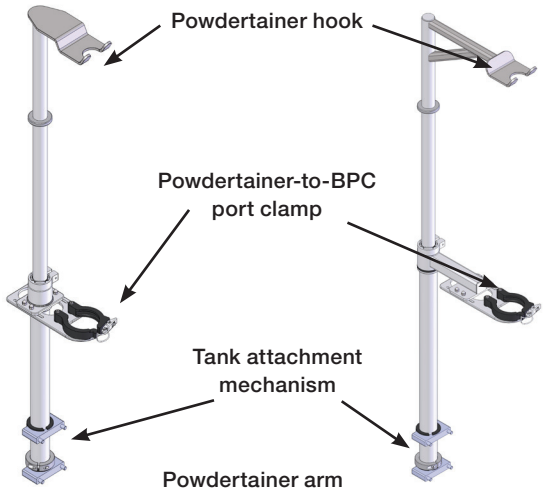
The optional cable management system connects to the left side of the S.U.M. and is used to properly route tubing and cables along the side of the S.U.M.

### Ordering information

| Description | Cat. No. |
|---|---|
| Cable management system (500 L–2,000 L) | SV50992.03 |

### Powdertainer arm

A Powdertainer arm is available as an option for powder-to-liquid applications. It holds the container of powder above the mixer and attaches it to the BPC with a clamp. The arm adjusts vertically and swivels to enable convenient lifting of the Powdertainer BPC onto the hanger.




### Ordering information

| Description | Cat. No. |
|---|---|
| Powdertainer arm (2,000 L) | SV51002.02 |

### Standard 2,000 L S.U.M. hardware

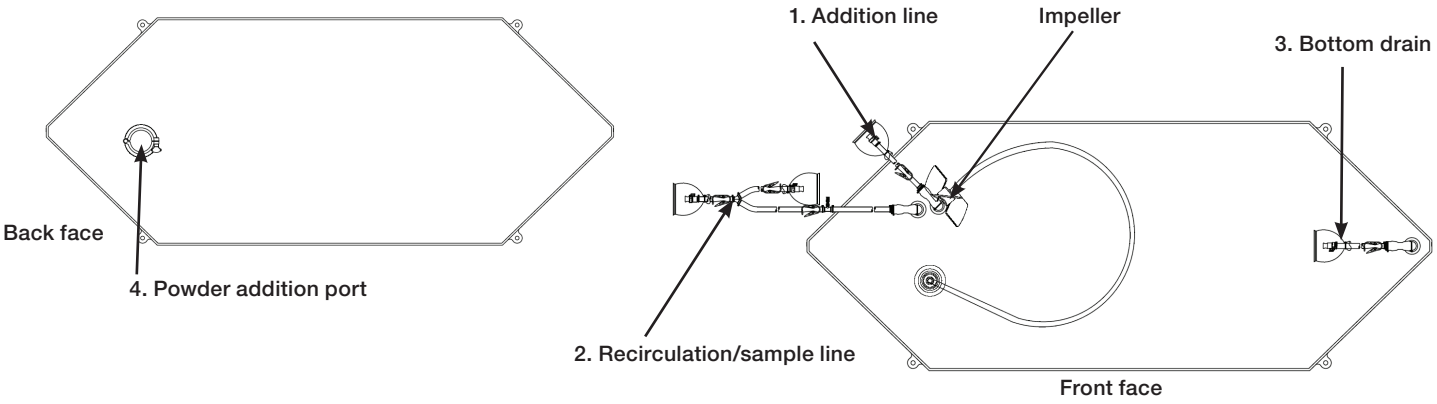
| 2,000 L S.U.M. specifications | | |
|---|---|---|
| | | Jacketed and non-jacketed |
| Mixer geometry | Rated liquid working volume | 2,000 L |
| | Minimum liquid working volume | 400 L (complete impeller coverage) |
| | Total chamber volume (liquid and gas) | 2,700 L |
| | BPC chamber diameter | 135 cm (53 in.) |
| | BPC chamber shoulder height | 185 cm (73 in.) |
| | Liquid height at rated working volume | 140 cm (55 in.) |
| | Fluid geometry at working volume (height:diameter ratio) | 1:1 |
| | Hold-up volume | <1 L |
| | Overall mixer geometry (height:diameter ratio) | 1.2:1 |
| | Tank baffles | None |
| Impeller | Impeller (quantity x blade count) | 1 x 3 |
| | Impeller scaling (impeller diameter:tank diameter) | 1:5 |
| | Impeller blade pitch (angle) | 45° |
| | Impeller diameter | 25.02 cm (9.85 in.) |
| Agitation | Mixing rate range | 20–356 rpm |
| | Tip speed | 39.4–459.7 cm/sec (77.6–904.8 ft/min) |
| | Counterclockwise mixing flow direction | Down-pumping |
| | Agitation shaft resolved angle | 27° |
| | Agitation shaft centerline offset | 17.8 cm (7 in.) |
| | Overall drive shaft length | 192.5 cm (75.78 in.) |
| | Drive shaft diameter | 1.9 cm (0.75 in.) |
| | Drive shaft poly-sheath outside diameter | 2.54 cm (1 in.) |
| | Impeller clearance from tank bottom | 5.08 cm (2 in.) |
| Motor | Agitation motor drive (type, voltage, phase) | Induction, 208 VAC, 3 phase |
| | Motor power rating | 745.7 W (1 hp)<br>Motor: 208 VAC, 3.2 A |
| | Motor torque rating | 18 Nm (159 in.-lb.) |
| | Gear reduction | 5:1 |
| | Motor communication methods | Via Touchscreen Console through communication ports |
| General | Ceiling height required for drive shaft loading | 292.1 cm (115 in.) |
| | Electrical power rating | 100–120 VAC, 50/60 Hz, single, 15 A<br>220–240 VAC, 50/60 Hz, single, 10.4 A |
| | pH & DO probe—autoclavable type<br>(Applisens, Broadley James, Mettler Toledo) | 12 mm diameter x 215–235 mm insertion length<br>x 13.5 PG (pipe) thread |
| | Noise level | <70 dB at 1.5 m |
| | Storage temperature | –25°C to 65°C |
| | Relative humidity | 20–80% non-condensing<br>AC motor: 100% max. (without any dew condensation) |
| Recommended operating parameters | Operating temperature range | S.U.M.: 2–40 ± 0.1°C (36–104 ± 0.2°F)<br>AC motor: 0–40°C (32–104°F) |
| | Motor speed | 30–350 rpm |
| | Volume range | 500–2,000 L |
| | Maximum static BPC pressure | 0.03 bar (0.5 psi) |
| | Maximum BPC pressure during operation | 0.007 bar (0.1 psi) |
| | Continuous operating time | 21 days mixing at nominal volume only |

## Standard 2,000 L S.U.M. hardware

| 2,000 L S.U.M. specifications | | | |
|---|---|---|---|
| | | Jacketed | Non-jacketed |
| Fluid jacket | Jacket area: full/half volume | 5.3 $m^2$/3.3 $m^2$ (57.3 $ft^2$/35.5 $ft^2$) | – |
| | Jacket volume | 26 L (6.9 gal) | – |
| | Jacket flow rate at 3.4 bar (50 psi) | 93 L/min (24.5 gal/min) | – |
| | Process connection | 1 in. sanitary tri-clamp | – |
| Temperature control | TCU model: maximum heating/cooling | TF24000: 22,500/24,000 W | – |
| | Approximate liquid heat-up time (5–37°C) | 2.7 hr | – |
| | Approximate liquid cool-down time (37–5°C) | 3.9 hr | – |
| | RTD or thermocouple, 3.18 mm (1/8 in.) OD | Pt-100 (standard) | Pt-100 (standard) |
| Support container (without shelves/ basket) | Overall width | 180 cm (70.7 in.) | 180 cm (70.7 in.) |
| | Overall length | 171 cm (67.4 in.) | 171 cm (67.4 in.) |
| | Overall height (without Powdertainer arm) | 225 cm (88.5 in.) | 225 cm (88.5 in.) |
| | Dry skid weight (mass) | 755.1 kg (1,664.8 lb.) | 551.1 kg (1,214.8 lb.) |
| | Wet skid weight—rated working volume (mass) | 2,755.1 kg (6,073.8 lb.) | 2,551.1 kg (5,623.8 lb.) |
| Touchscreen Console | Dimensions (W x H x D) | 250 x 520 x 234 mm (9.9 x 20.5 x 9.3 in.) | |
| | Construction material | AISI 304 (stainless steel) | |
| | Pumps | Supports various pumps with 4–20 mA signal control | |
| | Load cells | Mettler Toledo MTB | |
| | pH sensors supported | Thermo Scientific, Mettler Toledo, and Broadley James | |
| | Conductivity sensors | JUMO, Mettler Toledo | |
| | Pinch valves | Bimba ACRO 935 pinch valve,<br>3/4 in. (19 mm) OD x 1/8 in. (3.175 mm) wall tubing | |
| | HMI | 8.4 in. LCD panel with capacitive touchscreen | |
| | Alarms | Factory-set and user-defined | |
| | Communication ports | USB, Ethernet, Profibus, Modbus RTU | |
| | E-stop | Integrated safety circuit for entire system; external E-stop also available | |
| | Data recording | User-defined data record transfer via Ethernet, Profibus, or Modbus RTU.<br>72-hour data storage exportable via USB | |
| | File formats | CSV | |
| | Data exporting | Local via USB flash drive<br>Remote via PC and network with Ethernet, Profibus, or Modbus RTU | |

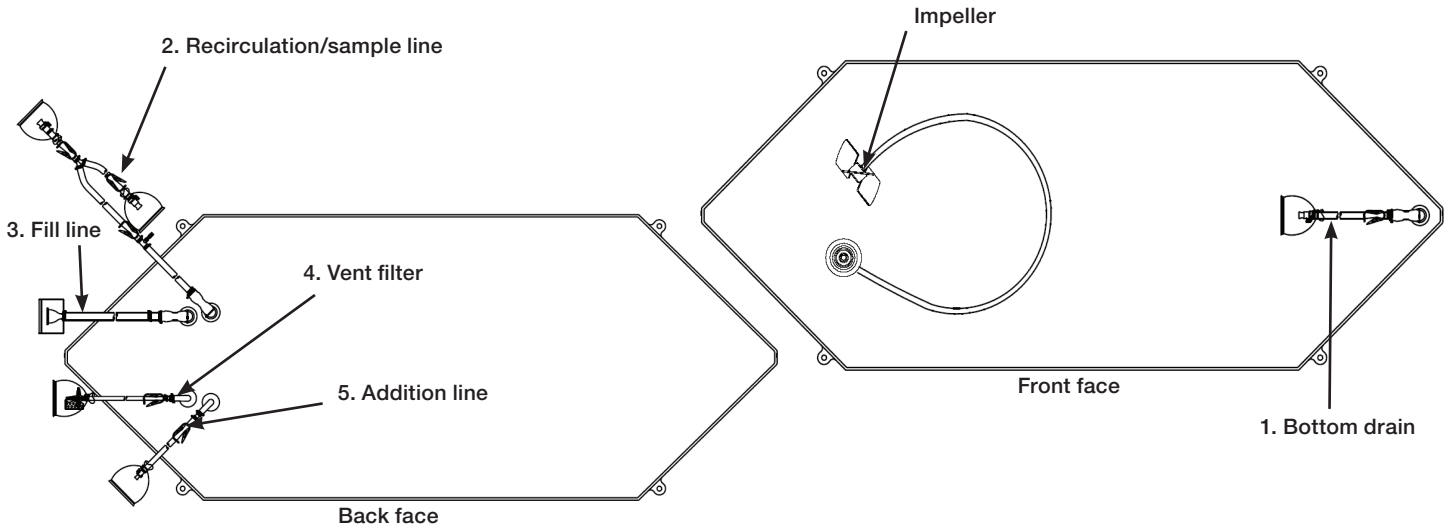
## HyPerforma S.U.M. BPCs

Open-top or closed-top Thermo Scientific™ BPC designs are available with Thermo Scientific™ CX5-14 and Aegis™ 5-14 film options.



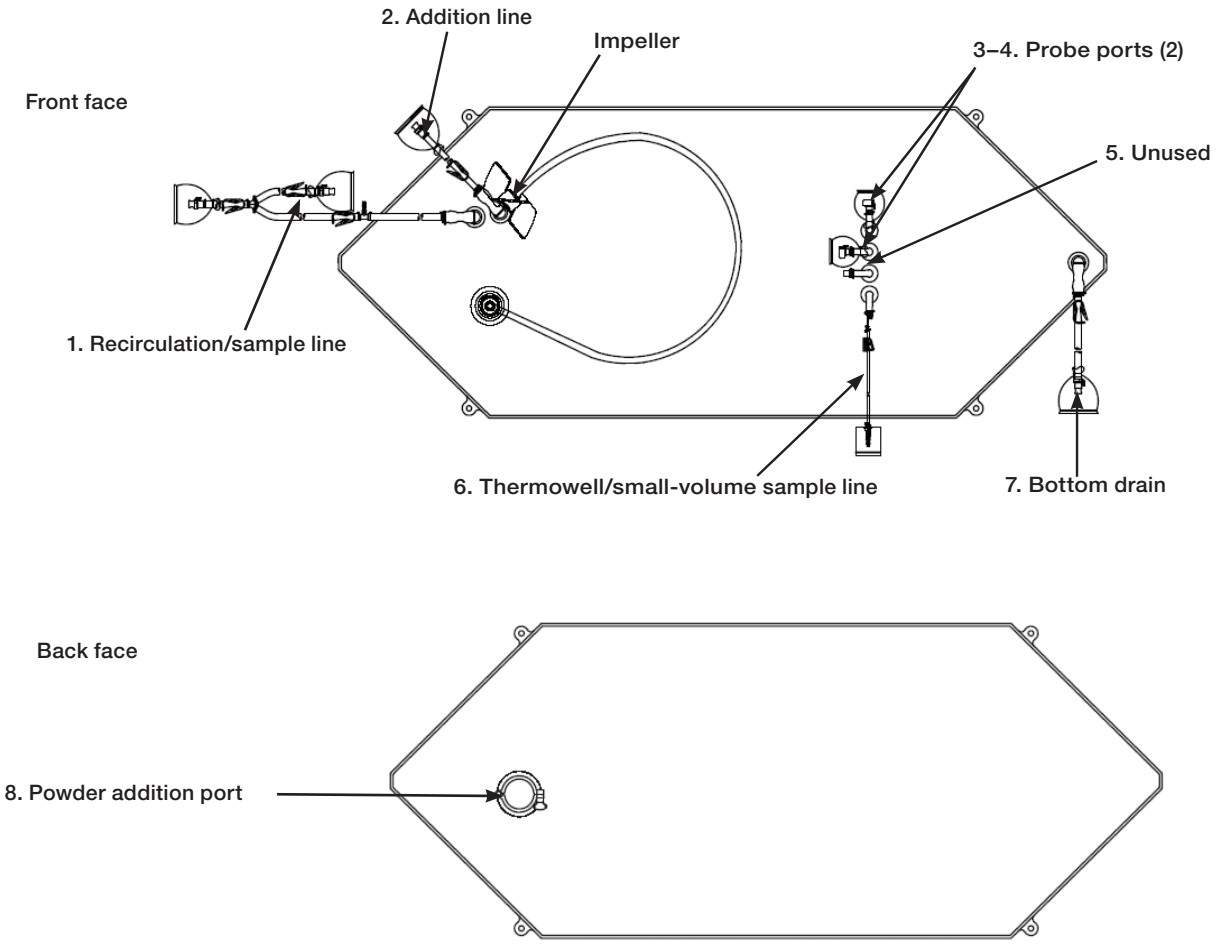

### Standard 2,000 L BPC for powder–to-liquid applications without probe ports

| Line | Description | Tubing set (inner diameter x outer diameter x length) | End treatment |
|---|---|---|---|
| 1 | Addition line | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 183 cm (72 in.) | Plugged 12.7 mm (1/2 in.) MPX body |
| 2 | Recirculation/sample line | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 198 cm (78 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 30 cm (12 in.) | Capped 12.7 mm (1/2 in.) MPX insert Plugged 12.7 mm (1/2 in.) MPX body |
| 3 | Bottom drain | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.) | Plugged 12.7 mm (1/2 in.) MPX insert |
| 4 | Powder addition port | 7.6 mm (3 in.) sanitary fitting, tri-clamp | Cap with gasket |



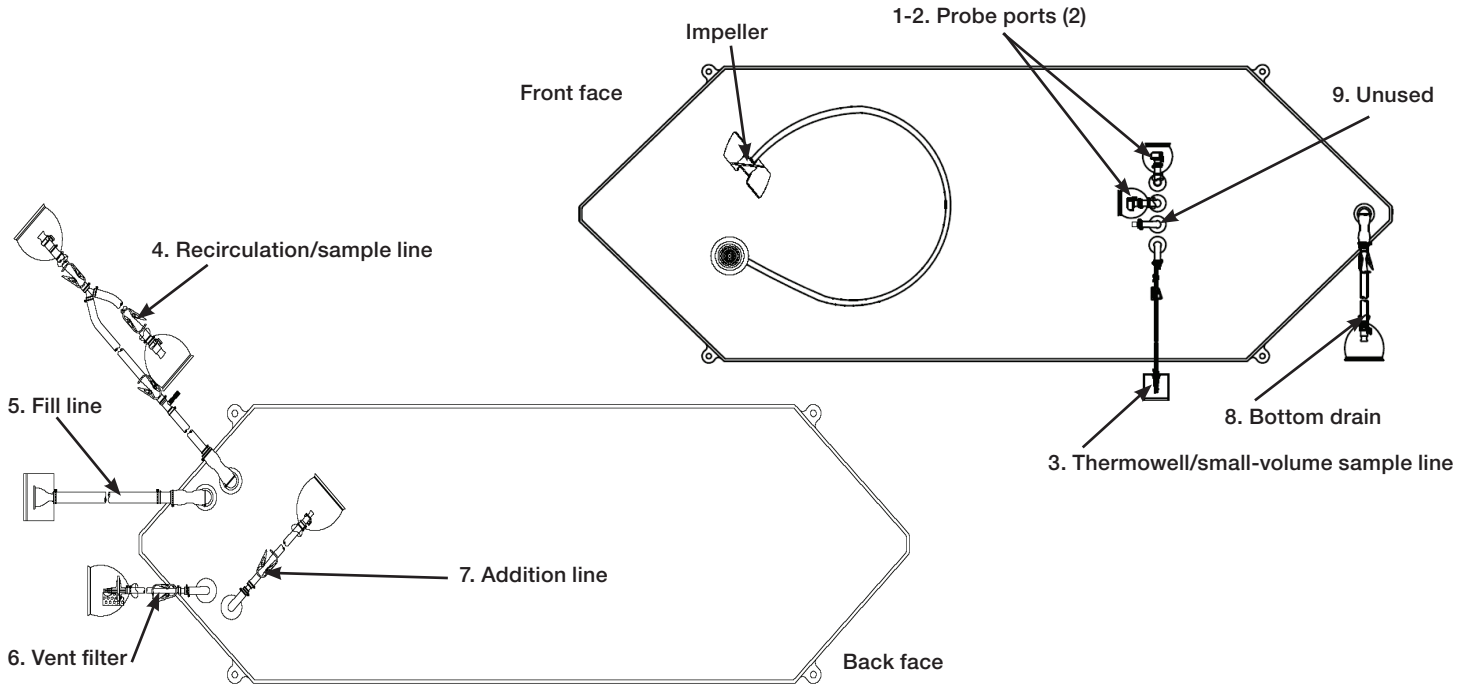

### Standard 2,000 L BPC for liquid-to-liquid applications without probe ports

| Line | Description | Tubing set (inner diameter x outer diameter x length) | End treatment |
|---|---|---|---|
| 1 | Bottom drain | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.) | Capped 12.7 mm (1/2 in.) MPX insert |
| 2 | Recirculation/ sample line | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 198 cm (78 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 30 cm (12 in.) | Capped 12.7 mm (1/2 in.) MPX insert Plugged 12.7 mm (1/2 in.) MPX body |
| 3 | Fill line | 19.1 mm (3/4 in.) x 25.4 mm (1 in.) C-Flex x 183 cm (72 in.) | 38.1 mm (1 1/2 in.) tri-clamp (SterilEnz™) |
| 4 | Vent filter | 6.4 mm (1/4 in.) x 12.7 mm (1/2 in.) C-Flex x 10.2 cm (4 in.) | Sterile hydrophobic vent filter (0.2 µm PVDF, Acro™ 50) |
| 5 | Addition line | 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex x 61 cm (24 in.) | Plugged 9.5 mm (3/8 in.) MPX body |



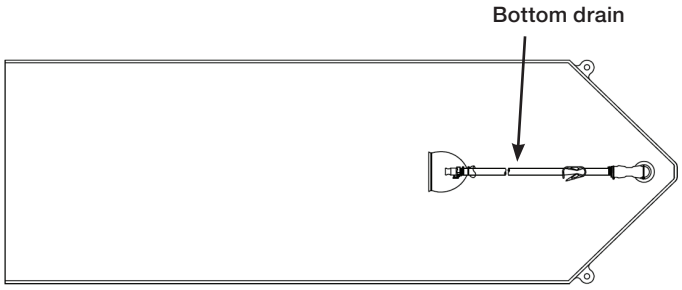

### Standard 2,000 L BPC for powder-to-liquid applications with probe ports

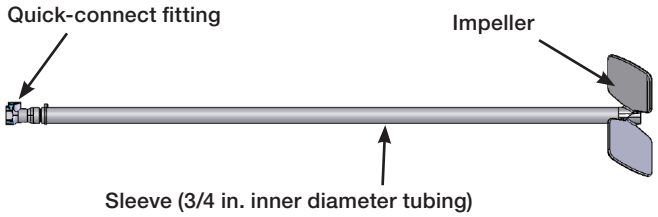
| Line | Description | Tubing set (inner diameter x outer diameter x length) | End treatment |
|---|---|---|---|
| 1 | Recirculation line | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 198 cm (78 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 30 cm (12 in.) | Capped 12.7 mm (1/2 in.) MPX insert<br>Plugged 12.7 mm (1/2 in.) MPX body |
| 2 | Addition line | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.) | Plugged 12.7 mm (1/2 in.) MPX insert |
| 3–4 | Probe ports (2) | None | Kleenpak™ aseptic connector KPCHT series (female) |
| 5 | Unused | None | Plug |
| 6 | Thermowell/ small-volume sample line | Thermowell adapter for 3.2 mm (1/8 in.) diameter 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) x 30 cm (12 in.) | Luer and SmartSite™ valve port |
| 7 | Bottom drain | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.) | Capped 12.7 mm (1/2 in.) MPX body |
| 8 | Powder addition port | 7.6 mm (3 in.) sanitary fitting, tri-clamp | Cap with gasket |




## Standard 2,000 L BPC for liquid–liquid applications with probe ports

| Line | Description | Tubing set (inner diameter x outer diameter x length) | End treatment |
|---|---|---|---|
| 1–2 | Probe ports (2) | None | Kleenpak aseptic connector KPCHT series (female) |
| 3 | Thermowell/ small-volume sample line | Thermowell adapter for 3.2 mm (1/8 in.) diameter 3.2 mm (1/8 in.) x 6.4 cm (1/4 in.) C-Flex x 30 cm (1/2 in.) | Luer and SmartSite valve port |
| 4 | Recirculation line | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 198 cm (78 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 30 cm (12 in.) | Capped 12.7 mm (1/2 in.) MPX insert Plugged 12.7 mm (1/2 in.) MPX body |
| 5 | Fill line | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 183 cm (72 in.) | 38.1 mm (1 1/2 in.) tri-clamp (SterilEnz) |
| 6 | Vent filter | 6.4 mm (1/4 in.) x 12.7 mm (1/2 in.) C-Flex x 10.2 cm (4 in.) | Sterile hydrophobic vent filter (0.2 µm PVDF, Acro 50) |
| 7 | Addition line | 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex x 61 cm (24 in.) | Plugged 9.5 mm (3/8 in.) MPX insert |
| 8 | Bottom drain | 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.) | Plugged 12.7 mm (1/2 in.) MPX body |
| 9 | Unused | None | Plug |




## Standard open-top liners

| Description | Cat. No. |
|---|---|
| 1,000 L standard open-top liner, 0 probes, CX3-9 film | SH30762.05 |

## Standard impeller sleeve

| Description | Cat. No. |
|---|---|
| 2,000 L impeller sleeve for open-top mixing* | SH30772.01 |

* The bearing hub needed for open-top mixing is automatically supplied with the tank hardware.

## Custom BPC products

| Category | Options/capability | Notes |
|---|---|---|
| Tubing type | C-Flex, platinum-cured silicone, PVC, PharMed™, PharmaPure™ | More information is available in the tubing selection guide |
| Tubing size | Ranges from 3.18 mm (1/8 in.) to 25.4 mm (1 in.) inner diameter in various lengths | More information is available in the tubing selection guide |
| Connectors | Luer, Colder Products Company™ (CPC) quick connects, SIP connectors, tri-clamp, Kleenpak, SmartSite, Clave™, Lynx™ steam-thru, CPC steam-thru, Gore™ steam valve, Gore™ Mini TC, BioQuate™, SterilEnz, end plug | More information is available in the connection system selection guide. Note: the only option for probe port connections is Kleenpak connectors |
| Probe ports/ line addition ports | Ports may be added if they are compatible with the hardware | The reusable probe port connection uses a Kleenpak connector |
| Disposable sensors | Pressure sensor: PendoTECH™ and Finesse Solutions (PendoTECH comes standard on 500 L and 1,000 L S.U.M.); DO and pH sensor: Finesse Solutions and PreSens™; pH sensor: Mettler Toledo | Choice of qualified vendors available |
| Port sizes | Limited engineer-to-order customization only | Dependent on location in BPC and fit with hardware (e.g., 1 in. ID port on harvest line) |
| Rearrangement of lines on existing ports | Limited customization possible, such as moving sample/thermowell port to a probe tube port, or swapping exhaust outlet line with liquid lines | Dependent on location in BPC and fit with hardware |
| Dip tube lines | Limited customization possible | Length cannot interfere with impeller and shaft |
| Filters on media and supplement inlets | Limited engineer-to-order customization only. Choice of filters used to sterilize incoming media or supplements are available | |

Note: Not all options are available for all ports. It is not possible to customize port type, port location, chamber dimensions, or mixing assembly.
For additional information, please see the selection guides in the product catalog.

## BPC packaging

| Description | Details |
|---|---|
| Outer packaging | Supplied flat-packed with two polyethylene outer layers |
| Label | Description, product code, lot number, and expiry date on outer packaging and shipping container |
| Sterilization | Irradiation (25 to 40 kGy) inside outer packaging |
| Shipping container | Durable cardboard carton |
| Documentation | Certificate of Analysis provided with each lot for each delivery |

thermo scientific

## Ordering information

| 2,000 L S.U.M. hardware | Cat. No. |
|---|---|
| Non-jacketed, AC motor, Touchscreen Console, 240 V, with load cells | SUM2000.9003 |
| Jacketed, AC motor, Touchscreen Console, 240 V, with load cells | SUM2000.9004 |

Models without water jackets may have slightly different dimensions than the water-jacketed model shown in this data sheet. See the drawings provided with your unit for exact dimensions for non-jacketed models. Non-jacketed models do not have the capability to heat or cool the liquid inside the tank.

| 2,000 L S.U.M. BPC | Size | Probe ports* | Film type | Cat. No. |
|---|---|---|---|---|
| Standard powder-to-liquid BPC | 2,000 L | 0 | CX5-14 | SH30770.01 |
| | 2,000 L | 0 | Aegis 5-14 | SH30973.05 |
| Standard liquid-to-liquid BPC | 2,000 L | 0 | CX5-14 | SH30769.01 |
| | 2,000 L | 0 | Aegis 5-14 | SH30983.05 |
| Standard powder-to-liquid BPC* | 2,000 L | 3 | CX5-14 | SH30770.02 |
| | 2,000 L | 3 | Aegis 5-14 | SH30974.05 |
| Standard liquid-to-liquid BPC* | 2,000 L | 3 | CX5-14 | SH30769.02 |
| | 2,000 L | 3 | Aegis 5-14 | SH30982.05 |

* All 2,000 L BPCs with probe ports are designed to allow probes to work properly at 5:1 turndown levels. These BPCs are only compatible with the HyPerforma hardware shown in this document. If you are using an older version of the S.U.M. hardware, do not use these items; instead refer to the legacy S.U.M. user manual or data sheets.

Find out more at **thermofisher.com/sum**

ThermoFisher SCIENTIFIC

Data Sheet

# Millipore Express® SHF Hydrophilic Filters

## High flow sterilizing-grade PES filters for validated process steps

Millipore Express® SHF (Sterile High-Flux) filters are an unparalleled combination of quality and process performance. A sterilizing-grade, hydrophilic polyethersulfone (PES) membrane, these filters provide sterility assurance, broad chemical compatibility, and exceptionally high flow rates for your validated process steps. Developed and designed for your validated sterile filtration processes, manufactured in a highly controlled process and supported by extensive documentation to fulfill your regulatory requirements.

## Benefits

- Highly efficient sterilizing-grade membrane
- Designed for validated filtration steps which require sterility assurance
  - Drug Master File available
  - Retention testing on each lot of membrane and filter device
  - Each filter subject to the highest sensitivity integrity test in the industry
  - Validation kits available

### Filter Formats

- OptiScale® small-scale disposable capsule filters
- Opticap® XL and XLT disposable capsule filters– autoclavable, sterile and gamma compatible
- Cartridge filters

### Sterilization Capability

- Autoclavable
- Sterile
- Gamma Compatible

EMD Millipore Corp. is a subsidiary of Merck KGaA, Darmstadt, Germany

## Regulatory Compliance

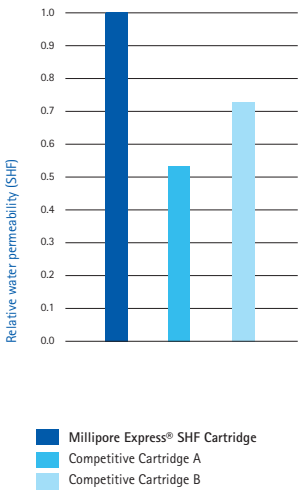
- Filters are designed, developed and manufactured in accordance with a Quality Management System approved by an accredited registering body to the ISO® 9001 Quality Systems Standard.
- Each filter and membrane lot is subjected to bacterial retention testing.
- Every filter is subjected to the highest sensitivity integrity test in the industry.
- All filters are shipped with a Certificate of Quality.
- A Validation Guide, which is a summary of the product qualification, is available upon request.
- For traceability and easy identification, each filter is identified with product name, lot number, and serial number.
- Drug Master File is 16877DMF

## Reduced Surface Area and Lower Costs

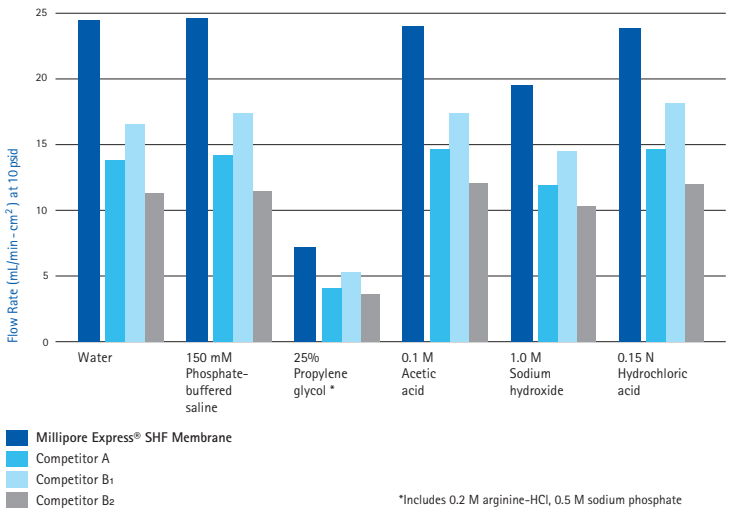
Millipore Express® SHF filters feature faster flow rates, allowing you to process equivalent volumes with reduced filtration area. This high-flux benefit has been shown to deliver greater than 50% savings in filtration costs.

# Superior Flux

### Relative Water Permeability of 10-inch Cartridges




### Millipore Express® SHF Membrane Flux Relative to Competitors




*Includes 0.2 M arginine-HCl, 0.5 M sodium phosphate

# Specifications

## Opticap® XL and XLT Disposable Capsules (Autoclavable)

| | Opticap® XL 3 Capsules | Opticap® XL 5 Capsules | Opticap® XL 10 Capsules | Opticap® XLT 10 Capsules | Opticap® XLT 20 Capsules | Opticap® XLT 30 Capsules |
|---|---|---|---|---|---|---|
| **Nominal Dimensions** | | | | | | |
| Maximum length: | 17.3 cm (6.8 in.) | 21.6 cm (8.5 in.) | 33.5 cm (13.2 in.) | 37.6 cm (14.8 in) | 62.5 cm (24.6 in) | 87.1 cm (34.3 in) |
| **Fitting to Fitting** | | | | | | |
| Sanitary flange to sanitary flange: | - | | | 15.2 cm (6.0 in.) | 15.2 cm (6.0 in.) | 15.2 cm (6.0 in.) |
| Sanitary flange to hose barb: | - | | | 17.5 cm (6.9 in.) | 17.5 cm (6.9 in.) | 17.5 cm (6.9 in.) |
| Hose barb to hose barb: | - | | | 19.8 cm (7.8 in.) | 19.8 cm (7.8 in.) | 19.8 cm (7.8 in.) |
| **Filtration Area** | 0.16 $m^2$ (1.7 $ft^2$) | 0.29 $m^2$ (3.1 $ft^2$) | 0.54 $m^2$ (5.8 $ft^2$) | 0.54 $m^2$ (5.8 $ft^2$) | 1.08 $m^2$ (11.6 $ft^2$) | 1.62 $m^2$ (17.4 $ft^2$) |
| **Materials of Construction** | | | | | | |
| Filter membrane: | Hydrophilic polyethersulfone | | | | | |
| Film edge: | Polypropylene | | | | | |
| Supports: | Polypropylene | | | | | |
| Structural components*: | Polypropylene | | | | | |
| Core: | Polysulfone | | | | | |
| Vent O-rings: | Silicone | | | | | |
| **Vent/Drain** | ¼ in. hose barb with double O-ring seal | | | | | |
| **Maximum Operating pressure** | 6900 mbar (100 psi) intermittent 23 C° | | | - | - | - |
| **Maximum Differential Pressure** | | | | | | |
| Forward: | 5500 mbar (80 psi) at 25 °C | | | | | |
| | 6900 mbar (100 psi) intermittent at 25 °C | | | | | |
| | 1000 mbar (15 psi) at 80 °C | | | | | |
| Reverse: | 2100 mbar (30 psi) intermittent at 25 °C | | | | | |
| **Bubble Point at 23 °C** | ≥ 4000 mbar (58 psi) air with water | | | | | |
| **Air Diffusion at 23 °C** | Through a water wet membrane at 2800 mbar (40 psi): | | | | | |
| | ≤ 9.1 cc/min. | ≤ 16.4 cc/min. | ≤ 30 cc/min. | ≤ 30 cc/min. | ≤ 60 cc/min. | ≤ 90 cc/min. |
| **Bacterial Retention** | Quantitative retention of $10^7$ CFU/$cm^2$ *Brevundimonas diminuta* ATCC® 19146 per ASTM® methodology. | | | | | |
| **Bacterial Endotoxin** | Aqueous extraction contains <0.25 EU/mL as determined by the Limulus Amebocyte Lysate (LAL) Test (per 10-inch filter). | | | | | |
| **TOC/Conductivity** | Autoclaved capsule effluent meets the WFI criteria for USP <643>, Total Organic Carbon and USP <645>, Conductivity, after a WFI water flush of: | | | | | |
| | 3.0 L | 5.5 L | 10 L | 10 L | 20 L | 30 L |
| **Oxidizable Substances** | Meets the USP Oxidizable Substances Test requirements for sterile purified water after a water flush of: | | | | | |
| | 1000 mL | 1000 mL | 1000 mL | 1000 mL | 2000 mL | 3000 mL |
| **Sterilization** | May be autoclaved for 3 cycles of 60 minutes at 126 °C. (Cannot be steam sterilized in-line). | | | | | |
| **Non-Fiber Releasing** | Component materials meet criteria for a "non fiber releasing" filter as defined in 21 CFR 210.3 (b) (6). | | | | | |
| **Component Material Toxicity** | Component materials were tested and meet the criteria of the USP <88> Reactivity Test for Class VI plastics. Millipore Express® SHF filters meet the requirements of the USP <88> Safety Test, utilizing a 0.9% sodium chloride extraction. | | | | | |
| **Toxicity** | Non-toxic per MEM Elution ISO® 10993-05. | | | | | |
| **Good Manufacturing Practices** | These products are manufactured in a facility which adheres to FDA Good Manufacturing Practices. | | | | | |
| **Indirect Food Additive** | All component materials meet the FDA Indirect Food Additive requirements cited in 21 CFR 177–182. | | | | | |

* Cage, end caps and capsule housing

# Specifications

## Opticap® XL and XLT Disposable Capsules (Sterile and Gamma Compatible)

| | Opticap® XL 3 Capsule | Opticap® XL 5 Capsule | Opticap® XL 10 Capsule | Opticap® XLT 10 Capsule | Opticap® XLT 20 Capsule | Opticap® XLT 30 Capsule |
|---|---|---|---|---|---|---|
| **Nominal Dimensions** | | | | | | |
| Maximum length: | 17.3 cm (6.8 in.) | 21.6 cm (8.5 in.) | 33.5 cm (13.2 in.) | 37.6 cm (14.8 in.) | 62.5 cm (24.6 in.) | 87.1 cm (34.3 in.) |
| Body diameter: | 10.7 cm (4.2 in.) | 10.7 cm (4.2 in.) | 10.7 cm (4.2 in.) | - | - | - |
| **Fitting to Fitting** | | | | | | |
| Sanitary flange to sanitary flange: | - | - | - | 15.2 cm (6.0 in.) | 15.2 cm (6.0 in.) | 15.2 cm (6.0 in.) |
| Sanitary flange to hose barb: | - | - | - | 17.5 cm (6.9 in.) | 17.5 cm (6.9 in.) | 17.5 cm (6.9 in.) |
| Hose barb to hose barb: | - | - | - | 19.8 cm (7.8 in.) | 19.8 cm (7.8 in.) | 19.8 cm (7.8 in.) |
| **Filtration Area** | 0.17 $m^2$ (1.8 $ft^2$) | 0.31 $m^2$ (3.3 $ft^2$) | 0.57 $m^2$ (6.1 $ft^2$) | 0.57 $m^2$ (6.1 $ft^2$) | 1.14 $m^2$ (12.3 $ft^2$) | 1.71 $m^2$ (18.4 $ft^2$) |
| **Materials of Construction** | | | | | | |
| Filter membrane: | Hydrophilic polyethersulfone | | | | | |
| Film edge: | Polyethylene | | | | | |
| Supports: | Polyester | | | | | |
| Structural components*: | Gamma stable polypropylene | | | | | |
| Core: | Polysulfone | | | | | |
| Vent O-rings: | Silicone | | | | | |
| **Vent/Drain** | ¼ in. hose barb with double O-ring seal | | | | | |
| **Maximum Differential Pressure** | | | | | | |
| Forward: | 5500 mbar (80 psi) at 25 °C | | | | | |
| | 6900 mbar (100 psi) intermittent at 25 °C | | | | | |
| | 1000 mbar (15 psi) at 80 °C | | | | | |
| Reverse: | 2100 mbar (30 psi) intermittent at 25 °C | | | | | |
| **Bubble Point at 23 °C** | ≥ 4000 mbar (58 psi) air with water | | | | | |
| **Air Diffusion at 23 °C** | Through a water wet membrane at 2800 mbar (40 psi): | | | | | |
| | ≤ 9.5 cc/min. | ≤ 17.4 cc/min. | ≤ 32.7 cc/min. | ≤ 32.7 cc/min. | ≤ 65.5 cc/min. | ≤ 98.2 cc/min. |
| **Bacterial Retention** | Quantitative retention of $10^7$ CFU/$cm^2$ *Brevundimonas diminuta* ATCC® 19146 per ASTM® methodology. | | | | | |
| **Bacterial Endotoxin** | Aqueous extraction contains <0.25 EU/mL as determined by the Limulus Amebocyte Lysate (LAL) Test (per 10-inch filter). | | | | | |
| **TOC/Conductivity** | Gamma sterilized capsule effluent meets the WFI criteria for USP <643>, Total Organic Carbon, and USP <645>, Conductivity, after a WFI water flush of: | | | | | |
| | 3.5 L | 6.0 L | 11 L | 11 L | 22 L | 33 L |
| **Oxidizable Substances** | Meets the USP Oxidizable Substances Test requirements for sterile purified water after a water flush of: | | | | | |
| | 1000 mL | 1000 mL | 1500 mL | 1500 mL | 3000 mL | 4500 mL |
| **Sterilization** | | | | | | |
| Gamma compatible: | Gamma compatible to 45 kGy. May be autoclaved for 3 cycles of 60 minutes at 123 °C. (Cannot be steam sterilized in-line). | | | | | |
| Sterile capsules: | May be autoclaved for 3 cycles of 60 minutes at 123 °C. (Cannot be steam sterilized in-line.) | | | | | |
| **Sterility** | | | | | | |
| **Sterile capsules:** | Meets current USP and AAMI guidelines for sterility utilizing a validated sterilization cycle. | | | | | |
| **Non-Fiber Releasing** | Component materials meet criteria for a "non fiber releasing" filter as defined in 21 CFR 210.3 (b) (6). | | | | | |
| **Component Material Toxicity** | Component materials were tested and meet the criteria of the USP <88> Reactivity Test for Class VI plastics. Millipore Express® SHF filters meet the requirements of the USP <88> Safety Test, utilizing a 0.9% sodium chloride extraction. | | | | | |
| **Toxicity** | Non-toxic per MEM Elution ISO® 10993-05. | | | | | |
| **Good Manufacturing Practices** | These products are manufactured in a facility which adheres to FDA Good Manufacturing Practices. | | | | | |
| **Indirect Food Additive** | All component materials meet the FDA Indirect Food Additive requirements cited in 21 CFR 177–182. | | | | | |

* Cage, end caps and capsule housing

Filters were tested post gamma radiation at 25-45 kGy

Millipore®
Filtration, Separation
& Preparation

Data Sheet

# QuikScale® Biochromatography Columns

## Innovative chromatography columns for scalable, rapid purification

Biopharmaceutical manufacturers increasingly require tools that maximize process yields, boost productivity, and shorten time-to-market. To meet these demands, the scalable family of QuikScale® columns delivers greater product purity at faster linear velocities than columns currently used in most pharmaceutical processes. As a part of our total solution, these innovative columns have been designed to be used easily with any biochromatography system, such as our K-Prime® systems, from small scale pilot processes through complex development and manufacturing operations.

### Ultra high throughput with all media types

Designed to achieve ultra high throughput, robust QuikScale® columns are easily, uniformly packed to deliver optimal resolution across a wide range of chromatographic applications, accommodating all media types. The innovative flow distributor assures full, uniform media utilization within the column, assuring reproducible and reliable separations. Using the high flow option with operating pressures up to 7 bar, flow rates up to 1000 cm/hr are achievable enabling greater process design flexibility by allowing greater bed heights when utilizing media with high flow and/or backpressure requirements.

**Benefits**

- High linear velocity for maximum productivity
- Easy to pack and unpack
- Rapid scale-up with a full range of column size from 70 mm to 630 mm
- Multiple tube materials that suit a wide variety of applications
- Maximum flexibility — change tube material and height in minutes



MilliporeSigma

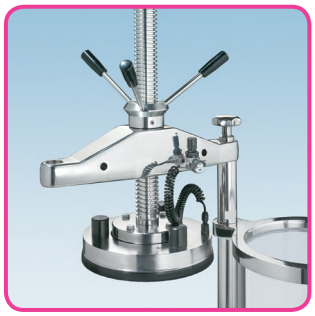
## Fast packing and unpacking

Column packing, processing, and unpacking are performed faster and with less effort. There's no heavy lifting or complex disassembly involved; all components remain attached to the column. An optional removable lower end-cell makes unpacking simple and quick. The simple design eliminates the need for tools and hoists during packing and unpacking.

A yoke and central screw mechanism supports the flow distributor, ensuring this assembly easily moves during packing and unpacking. The flow distributor makes it easy to pack uniform beds. The flow distributor can be swiveled to afford easy access for maintenance without the need to lift the adjuster assembly from the column.

## Flow distributor seal technology ensures reliable, fail-safe operation

To make packing simple and efficient, the seal (patent pending) is spring force actuated and uses air to deactivate the seal. This design offers smooth vertical movement, and allows the flow distributor assembly to be gently positioned and secured, without disrupting the packed bed.



The bed support, which is available for 10 µm, 20 µm and 30 µm sizes, consists of stainless steel mesh screens that enhance flow distribution efficiency. The screens, unlike plastic supports, allow the passage of air without requiring pre-wetting.

## Predictable, scalable performance

Column materials and geometries were carefully chosen to ensure consistent chromatographic performance from lab scale to production. QuikScale® columns are available in nine diameters from 70 mm to 630 mm. Stainless steel mesh screens, flow cells and column tubes for each column diameter are made of the same materials and are designed to ensure reliable performance across the range of column diameters. Column materials, a unique seal design, and optimized flow distribution, all contribute to creating predictable, scalable processes.

## Choice of three column tube materials, for any application

QuikScale® columns meet a broad range of applications. Tubes are offered in three materials:

- Cast acrylic, resistant to breakage with improved ethanol resistance and low protein binding
- Glass, suitable for a wide range of process application
- Stainless steel for improved corrosion resistance and high cleanability

All materials used in the manufacture of the column that come into contact with the process stream conform to relevant sections of the FDA Code of Federal Regulations Volume 21, parts 170 to 199, or have passed USP Class VI tests for toxicity.

## Dependable construction for long-lasting, reliable performance

Durable frame design and construction, as well as robust tube materials ensure excellent physical stability to deliver years of trouble-free operation. Specially developed tube manufacturing technology results in excellent tube quality on a consistent basis. This reliability, throughout the QuikScale® column product line, is important for generating reproducible performance critical in pharmaceutical manufacturing.

## Modular design

A full line of accessories and installation kits enables quick set-up. Versatile configurations make it easy to adapt columns for use in multiple process applications.

**QuikScale® Chromatography**
Columns Featuring Industry Leading Chromatography Resin

### Eshmuno® Resin

Unique family of ion-exchange resins specifically designed for highly productive downstream purification.

### Fractogel® Resin

Tentacle modified synthetic polymer resins for ion exchange, hydrophobic interaction, size exclusion and metal chelate affinity.

### ProSep® Ultra Plus Resin

The highest dynamic binding capacity protein A affinity chromatography media, designed for cost effective, large-scale purification of today's higher titer therapeutic antibodies.

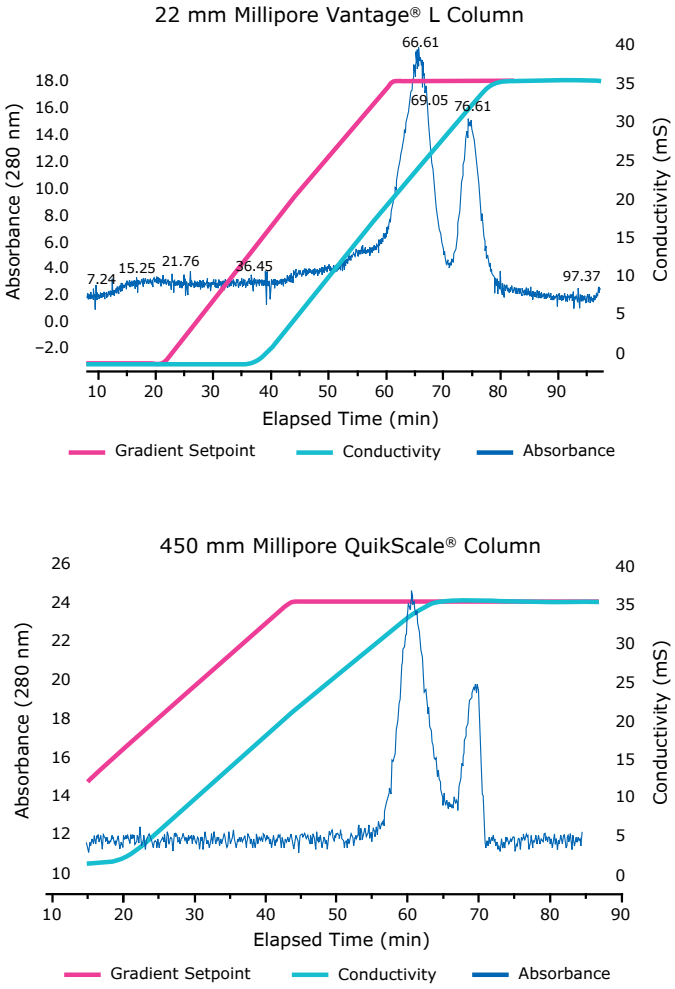



**Figure 1. Quick and reliable scale-up**

Separation of ovalbumin from trypsin inhibitor was scouted on a Millipore Vantage® L column (22 mm). The process was then linearly scaled up (418X by volume, 20X by diameter) to a 450 mm QuikScale® column with highly comparable results.

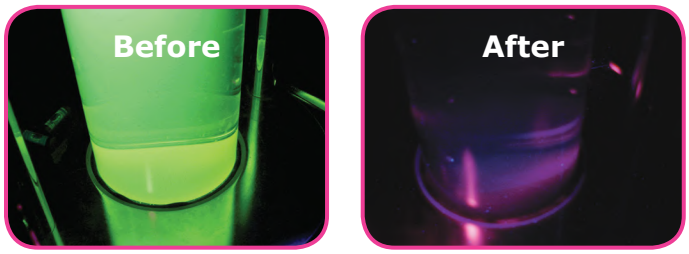



**Figure 2. Before and after images of riboflavin presence (green) in a 100 mm QuikScale® column**

Glass 100 mm and acrylic 450 mm Quikscale® columns were challenged with a concentrated riboflavin solution and subsequently cleaned with 0.5 M NaOH.

## Chemical resistance of QuikScale® Columns*

| Substance | Column | | |
|---|---|---|---|
| | Glass | Acrylic | Steel |
| Acetic acid (80%) | R | NR | R |
| Acetone | R | L | R |
| Acetonitrile | R | NR | R |
| Ammonium sulphate (10 – 40%) | R | R | R |
| Disodium phosphate | R | R | R |
| Ethanol (40%) | R | R | R |
| Ethylene glycol | R | R | R |
| Formaldehyde (50%) | R | R | R |
| Isopropanol | R | L | R |
| Methanol | R | L | R |
| Nitric acid (10%) | R | R | R |
| Peracetic acid (300 ppm) | L | L | L |
| Phosphoric acid (55%) | R | R | R |
| Potassium chloride (30%) | R | R | R |
| Potassium hydroxide (2M) | R | R | R |
| Sodium bicarbonate (20%) | R | R | R |
| Sodium chloride (2M) | R | R | R |
| Sodium hydroxide (2M) | L | L | L |
| Sodium hypochlorite (200 ppm) | L | L | L |
| Triton® X-100 surfactant | R | R | R |
| Urea (8M) | R | R | R |

R = Resistant; suitable for continuous use

L = Limited resistance; depends on concentration and contact time

NR = Not resistant, not recommended

* All substances are at 100% concentration (saturated solutions) unless noted otherwise. Concentrations: % refer to w/w. All data is referenced to room temperature (15 °C – 25 °C). A more extensive and detailed list is available in the product manual.

## Simple and convenient to clean and operate

QuikScale® columns are designed for clean-in-place (CIP), low maintenance, and sanitary operation. Smooth surface finish minimizes entrapment of contaminants and prevents corrosion. The elimination of O-rings in product contact areas prevents unswept surfaces, providing better cleanability.

A riboflavin clearance test performed on QuikScale® columns demonstrated that they can be effectively sanitized. Glass 100 mm and acrylic 450 mm Quikscale® columns were challenged with a concentrated riboflavin solution and subsequently cleaned with 0.5 M NaOH. The observable removal of riboflavin (Figure 2) indicates that there are no dead flow areas within the Quikscale® columns and that all areas are swept with sufficient and consistent velocity.

## Ordering information

### QuikScale® Columns – GA and GS Range

The QuikScale® column is available in two different configurations. For applications that require frequent packing and unpacking, the column may be ordered with a removable end cell (GA) and optional transport dolly. For applications that require infrequent packing a fixed bottom end cell is available (GS).

| Standard QuikScale® Columns | | | Catalogue Number | | |
|---|---|---|---|---|---|
| Column Diameter (nominal) | Material | Pressure Rating (bar) | Tube Length | | |
| | | | 550 mm | 800 mm | 1100 mm |
| 70 mm | Acrylic | 7 | GA071711 | GA071712 | GA071713 |
| | | | GS071711 | GS071712 | GS071713 |
| | Glass | 7 | GA072711 | GA072712 | GA072713 |
| | | | GS072711 | GS072712 | GS072713 |
| 100 mm | Acrylic | 7 | GA101711 | GA101712 | GA101713 |
| | | | GS101711 | GS101712 | GS101713 |
| | Glass | 7 | GA102711 | GA102712 | GA102713 |
| | | | GS102711 | GS102712 | GS102713 |
| 140 mm | Acrylic | 7 | GA141711 | GA141712 | GA141713 |
| | | | GS141711 | GS141712 | GS141713 |
| | Glass | 6 | GA142611 | GA142612 | GA142613 |
| | | | GS142611 | GS142612 | GS142613 |
| 200 mm | Acrylic | 7 | GA201711 | GA201712 | GA201713 |
| | | | GS201711 | GS201712 | GS201713 |
| | Glass | 4.8 | GA202511 | GA202512 | GA202513 |
| | | | GS202511 | GS202512 | GS202513 |
| 250 mm | Acrylic | 5 | GA251511 | GA251512 | GA251513 |
| | | | GS251511 | GS251512 | GS251513 |
| | Stainless | 5 | GA253511 | GA253512 | GA253513 |
| | | | GS253511 | GS253512 | GS253513 |
| 300 mm | Acrylic | 5 | GA301511 | GA301512 | GA301513 |
| | | | GS301511 | GS301512 | GS301513 |
| | Glass | 3 | GA302311 | GA302312 | GA302313 |
| | | | GS302311 | GS302312 | GS302313 |
| | Stainless | 5 | GA303511 | GA303512 | GA303513 |
| | | | GS303511 | GS303512 | GS303513 |
| 350 mm | Acrylic | 5 | GA351511 | GA351512 | GA351513 |
| | | | GS351511 | GS351512 | GS351513 |
| | Stainless | 5 | GA353511 | GA353512 | GA353513 |
| | | | GS353511 | GS353512 | GS353513 |
| 450 mm | Acrylic | 5 | GA451511 | GA451512 | GA451513 |
| | | | GS451511 | GS451512 | GS451513 |
| | Stainless | 5 | GA453511 | GA453512 | GA453513 |
| | | | GS453511 | GS453512 | GS453513 |
| 630 mm | Acrylic | 3 | GA631321 | GA631322 | — |
| | Stainless | 3 | GA633321 | GA633322 | — |

## Spare parts and accessories

The QuikScale® columns are complemented by a range of spare parts and accessories, including:

- High flow kit
- Process valves
- Connection kits
- Seals, clamps and adapters
- Bubble traps
- Column tube kits
- Replacement parts

## Support for your application needs

We have the know-how to provide you with everything you need for your process development applications. Technical briefs describe the benefits you will acquire when using the QuikScale® columns. For more information, call our Technical Service department or ask your Application Specialists for in-depth technical notes. An operator's manual contains full details of components, packing, testing, procedures for cleaning and sanitizing, troubleshooting and the spare parts list.

## To place an order or receive technical assistance

In the U.S. and Canada, call toll-free
1-800-645-5476

For other countries across Europe and the world, please visit:
emdmillipore.com/offices

For Technical Service, please visit:
emdmillipore.com/techservice

**milliporesigma.com**



Lit. No. DS1224EN00 Ver 6.0
2016 - 00659
02/2017

MILLIPORE

# Viresolve®
## Prefilter

### Custom protection for Viresolve NFP viral clearance filters

- Increases NFP capacity reducing overall filtration costs
- Improves robustness by decreasing the impact of feed variability on the NFP filter
- Maintain high product yields
- Simple, predictable scale-up and scale-down

The Viresolve Prefilter improves robustness and filtration economics of the viral clearance step by protecting Viresolve NFP viral clearance filters and decreasing the impact of feed stream variability. Use of this prefilter with NFP will improve capacity and increase the life of the NFP filter. Available in scalable filter formats, this prefilter will easily fit in existing development and manufacturing processes.

### Improved Robustness

Use of the prefilter improves the robustness of the filtration step by decreasing the impact minor changes in feed stream quality, such as batch-to-batch variability, effect of hold time, and freeze/thaw, etc., could have on the Viresolve NFP filter.

### Increased Capacity and Filter Life

Utilizing this prefilter in series with Viresolve NFP filters (NFP) will allow you to take advantage of NFP's high flow rates and product yield. The prefilter effectively removes fouling components of the feed, leading to dramatic increases in the performance of the Viresolve NFP filter. This improved capacity significantly reduces the cost of the viral clearance step.

**Table 1.** Example of Cost Savings with Prefiltration

Economic Evaluation of Prefilter Benefits for a Low-Capacity Feed Stream Utilizing Viresolve NFP Filters

| Process Description | Without Prefilter | With Prefilter |
|---|---|---|
| Feed volume | 500 L | 500 L |
| Viresolve NFP capacity | 60 L/m$^2$ | 300 L/m$^2$ |
| Viresolve prefilter sizing | — | 600 L/m$^2$ |
| Cost of viral clearance filter | $54,000 | $10,000 |
| Cost of Viresolve Prefilter | — | $ 400 |
| **Total Cost** | **$54,000** | **$10,400** |

### Filter Formats

- OptiScale®-40 disposable capsule filters
- Opticap® disposable capsule filters
- Pod disposable devices

## Custom Prefiltration for Viresolve NFP Filters

Viresolve NFP filters clear parvovirus from therapeutic feed streams without compromising flow rates, adding speed and dependability to viral clearance in therapeutic drug manufacturing applications. These reliable filters are ideal for monoclonal antibody polishing applications eliminating small virus contaminants.

## Regulatory Compliance

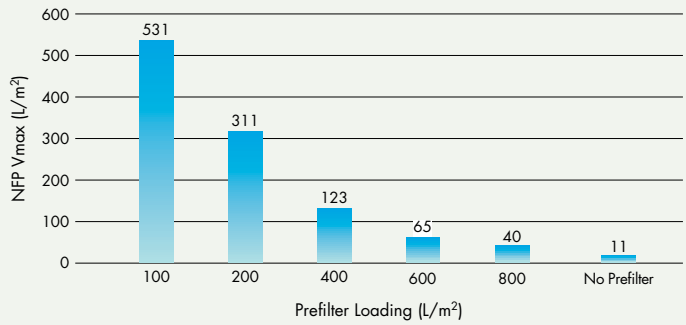
Viresolve Prefilters are designed, developed, and manufactured in accordance with good manufacturing practices under an ISO® 9001 Quality Management System. Opticap capsules and Pods are integrity tested during manufacturing and supported by a Validation Guide to assist in compliance with regulatory requirements. For traceability and easy identification, each filter is labeled with the product name and identifying characteristics. Every filter is shipped with a Certificate of Quality.

Table 2. Viresolve Prefilter Provides High Protein Yield

### Yield Analysis Summary with OptiScale-40 Devices

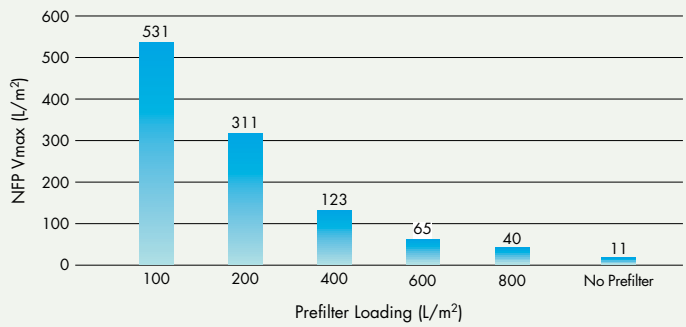
| Source | Recovered Mass |
|---|---|
| Filtrate | 94.5% |
| Flush | 2.2% |
| System hold-up loss | 2.5% |
| **Total** | **99.2%** |

Figure 1. Protection of Viresolve NFP Filter Increases Capacity




*Human IgG (1 g/L, pH 7.2) was processed through the Viresolve Prefilter at varying loading level. The filtrate from the prefilter was filtered through an OptiScale-25 capsule with Viresolve NFP membrane and the capacity of the NFP filter was measured.*

Figure 2. Viresolve NFP $V_{max}$ Capacity for Prefilter Scale-up




*Human IgG (1 g/L, pH 7.2) was filtered through 3 lots of OptiScale-40 capsules with Viresolve Prefilter media, and 2 lots of 10-inch Opticap capsules with Viresolve Prefilter media. The filtrates were then filtered through an OptiScale-25 with Viresolve NFP membrane and the filter capacity of the NFP was measured.*

# *From process development to full-scale production, Millipore has the right solution for you!*

## OptiScale-40 Small Volume Disposable Capsule Filters

*OptiScale-40 Filters*

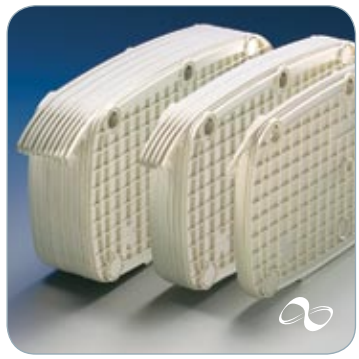
OptiScale-40 disposable capsule filters provide an active filtration area of 5 $cm^2$. These small devices are useful for process development and optimization, and viral clearance studies. A female Luer–Lok fitting/male Luer slip connection ensures fast and secure setup. These capsules are used in small volume applications where feedstock requirements are minimal.

## Opticap Disposable Capsule Filters

*Opticap Capsule Filters*

Opticap disposable capsule filters feature a patented design that withstands high thermal and hydraulic stress, assuring sterilization compatibility.

## Pod Disposable Devices



*Pod Filters*

Scalable from pilot to process applications, this new innovative NFF device format offers process flexibility and linear scale-up.

### Flexible Configurations

This patented technology consists of three Pod sizes and an expandable holder system.

Pod Prefilters are offered in 0.11 $m^2$, 0.55 $m^2$ and 1.1 $m^2$ filtration areas.

Two basic holders are available; the pilot scale holder is configurable with extension rods that can accommodate up to five 1.1 $m^2$ Pods, while the process scale holder expands to mount from 5 to 30 Pod devices. The stainless steel holder is not wetted by product and the connectors are disposable plastic. With the compact, modular design of Millipore's new pod system, you can increase productivity, shorten cycle-time and reduce costs.

**A Mobius technology.**

Mobius solutions feature a range of disposable technologies and services to optimize biopharmaceutical processes.

mobius™
flexible bioprocessing solutions

# Specifications

| | OptiScale-40 | 10 in. Opticap | 0.11 $m^2$ Pod | 0.55 $m^2$ Pod | 1.1 $m^2$ Pod |
|---|---|---|---|---|---|
| **Effective Filtration Area** | 5.0 $cm^2$ | 850 $cm^2$ | 0.11 $m^2$ | 0.55 $m^2$ | 1.1 $m^2$ |
| **Materials of Construction** | | | | | |
| *Media:* | Cellulose fibers with inorganic filter aid | Cellulose fibers with inorganic filter aid | Cellulose fibers with inorganic filter aid | | |
| *Membrane:* | Mixed esters of cellulose | Mixed esters of cellulose | Mixed esters of cellulose | | |
| *O-ring:* | — | Silicone | — | | |
| *Flat seal:* | — | — | Thermoplastic elastomer | | |
| *Housing:* | Polypropylene | Polypropylene | Glass-filled polypropylene | | |
| **Standard Connections** | Female Luer-Lok, male luer slip fittings | 3/4 in. Sanitary flange | Disposable 1 1/2 in. TC fitting | | |
| **Vent/Drain** | — | 1/8 in. Hose barb | Disposable vent | | |
| **Maximum Operating Line Pressure (at 23 °C)** | 4.1 bar (60 psi) | 5.5 bar (80 psi) | 3.4 bar (50 psi) | | |
| **Maximum Differential Pressure (at 23 °C)** | | | | | |
| *Forward:* | 2.1 bar (30 psi) | 2.1 bar (30 psi) | 2.1 bar (30 psi) | | |
| *Reverse:* | 0.03 bar (0.5 psi) | 0.35 bar (5 psi) | 2.1 bar (30 psi) | | |
| **Autoclaving** | May be autoclaved for 3 cycles of 60 minutes at 123 °C. | May be autoclaved for 3 cycles of 60 minutes at 123 °C. | May be autoclaved for 3 cycles of 60 minutes at 123 °C. | | |
| **TOC/Conductivity** | — | Lot release testing on effluent exhibited TOC <3 ppm and conductivity <10 µS/cm after autoclaving and a water flush of 10 L/$ft^2$. | Lot release testing on effluent exhibited TOC <4 ppm and conductivity <10 µS/cm after 3 autoclave cycles and a water flush of 100 L/$m^2$. | | |
| **Metals** | — | Lot release testing on effluent exhibited the following values:<br>Pb < 0.01 mg/$ft^2$<br>Hg < 0.01 mg/$ft^2$<br>As < 0.01 mg/$ft^2$<br>Fe < 0.1 mg/$ft^2$<br>Al < 0.5 mg/$ft^2$ | | Pb < 0.01 mg/$ft^2$<br>Hg < 0.01 mg/$ft^2$<br>As < 0.012 mg/$ft^2$<br>Fe < 0.1 mg/$ft^2$<br>Al < 0.5 mg/$ft^2$ | |
| **Bacterial Endotoxin** | Aqueous extraction contains <0.25 EU/mL as determined by the Limulus Amebocyte Lysate (LAL) test. | | | | |
| **Component Material Toxicity** | Component materials were tested and meet the criteria of the USP <88> Reactivity Test for Class VI Plastics. Viresolve Prefilters meet the requirements of the USP <88> Safety Test, utilizing a 0.9% sodium chloride extraction. | | | | |
| **Indirect Food Additive Quality Standard** | All component materials meet the FDA Indirect Food Additive requirements cited in 21 CFR 177–182. These products are manufactured in accordance with a Quality Management System that is approved by an accredited registering body to the appropriate ISO 9000 Quality Systems Standard. | | | | |

# Ordering Information

| Device | Nominal Process Volume | Connections | Qty/Pk | Catalogue No. |
|---|---|---|---|---|
| OptiScale-40 Capsule | 100 mL | Female Luer-Lok, male luer slip fittings | 9 | SSPV A40 NB9 |
| 10 in. Opticap Capsule | 20 – 40 L | 3/4 in. Sanitary flange inlet and outlet | 1 | KSPV 01F F1 |
| 0.11 $m^2$ Pod Filter | 20 – 40 L | Disposable 1 1/2 in. TC inlet and outlet | 1 | MSPV 01 FS1 |
| 0.55 $m^2$ Pod Filter | 100 – 200 L | Disposable 1 1/2 in. TC inlet and outlet | 1 | MSPV 05 FS1 |
| 1.1 $m^2$ Pod Filter | 200 – 400 L | Disposable 1 1/2 in. TC inlet and outlet | 1 | MSPV 10 FS1 |

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Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Data Sheet

# Viresolve® Pro Solution

## Proven viral safety solution designed to provide the highest levels of retention assurance and productivity

### Robust. Productive. Proven.

The Viresolve® Pro Solution provides a comprehensive, flexible template solution for virus filtration in biologics manufacturing. This proven viral clearance solution delivers the highest levels of retention assurance and processing efficiency across a broad range of feed streams.

The Viresolve® Pro Solution is comprised of the innovative, high-performing Viresolve® Pro Device in conjunction with the Viresolve® Pro Shield or the Viresolve® Pro Shield H prefilters. These products are designed to work togther to meet your needs providing high parvovirus retention, capacity and flux. Our industry-leading products and services, coupled with our viral clearance expertise, will help you successfully develop, implement, and validate the Viresolve® Pro Solution.

### Benefits

#### Viresolve® Pro Solution: High-productivity Virus Filtration

- Improved process economics with high mass capacity
- High flux for faster processing
- Consistent batch-to-batch performance
- Easy to install, use and integrity test
- Caustic sanitizable

#### Viresolve® Pro Device: Robust Parvovirus Clearance

- ≥ 4.0 log removal of parvovirus
- Devices are 100% integrity tested with air/water diffusion and Binary Gas
- Robust retention maintained during process interruption/depressurization



MilliporeSigma

## Prefilters: Viresolve® Pro Shield & Viresolve® Pro Shield H

Provides robust adsorptive (cation/mixed-mode) prefiltration to remove fouling protein aggregates

- Enhance throughput and process robustness of Viresolve® Pro Devices
- Effective across a broad range of pH and conductivity conditions

### Enhanced Process Robustness

For feed streams with high levels of fouling protein aggregates, the Viresolve® Pro Shield or Viresolve® Pro Shield H can be used to improve the capacity of the Viresolve® Pro Device.

These membrane prefilters adsorb protein aggregates that foul or plug the pores in the Viresolve® Pro membrane. Viresolve® Pro Shield and Viresolve® Pro Shield H have different membrane surface modifications to maximize adsorption of protein aggregates under a broad range pH and conductivity conditions (Figure 1).

The Viresolve® Pro Prefilter Selector Guide (TB1140EN00) provides guidance and information on prefilter selection.

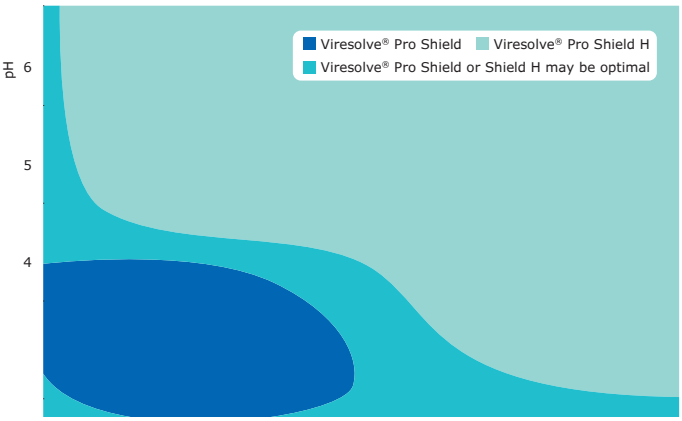
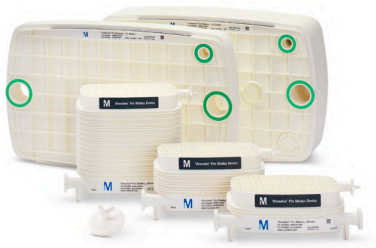



**Figure 1.**
Contour plot showing the optimal pH and conductivity conditions for Viresolve® Pro Shield and Viresolve® Pro Shield H.

## Virus Filter: Viresolve® Pro Device



Provides robust viral clearance

- ≥ 4.0 logs of Minute Virus of Mice clearance
- ≥ 5.0 logs of Murine Leukemia Virus clearance
- Delivers high capacity

### High Virus Retention

Retention performance of the Viresolve® Pro Micro 40 Devices, containing two lots of membrane, was evaluated under aggressive processing conditions. Testing was performed with a monoclonal antibody feed stream at 60 psi to a filtration endpoint of 90% flow decay, followed by a 20 L/m$^2$ buffer flush. Samples were collected from final filtrate pools and a summary of calculated log reduction values (LRV) is shown in Figure 2. As can be seen from the results, the Viresolve® Pro Solution achieved at least 5.8 logs of MVM retention. These results demonstrate robust virus clearance, even out to 90% flow decay, at high pressure.

Virus retention performance of the Viresolve® Pro Solution under a range of processing conditions is summarized in the application note *Virus Retention Performance of Viresolve® Pro Devices under a Range of Processing Conditions* (WP3374EN).

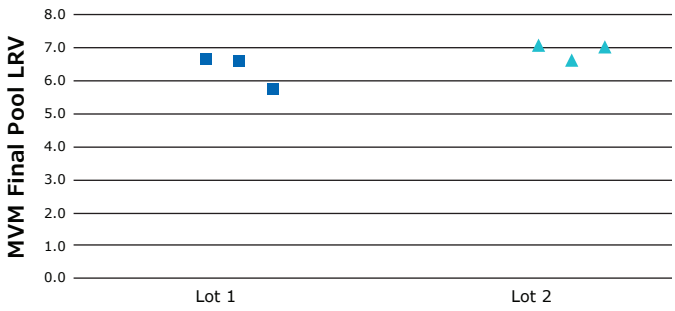


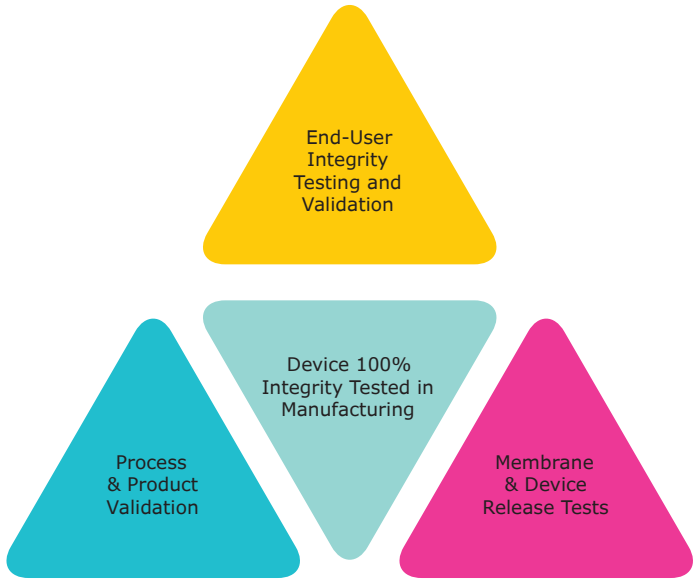

**Figure 2.**
Summary of MVM results for two membrane lots of Viresolve Pro® Micro 40 Devices.

## Building Quality

The virus retention performance and integrity of Viresolve® Pro Devices is assured with our comprehensive approach to quality.

### Assuring Retention

Retention performance is assured with our proprietary Binary Gas test which detects defects as small as 3-5 microns, that cannot be detected using a traditional air/water diffusion test, Figure 3. This high sensitivity test is especially valuable for Viresolve® Pro Devices, where small defects could impact virus retention performance. Every Viresolve® Pro Device must pass Binary Gas testing before release, assuring the highest levels of virus retention for your filtration operations.



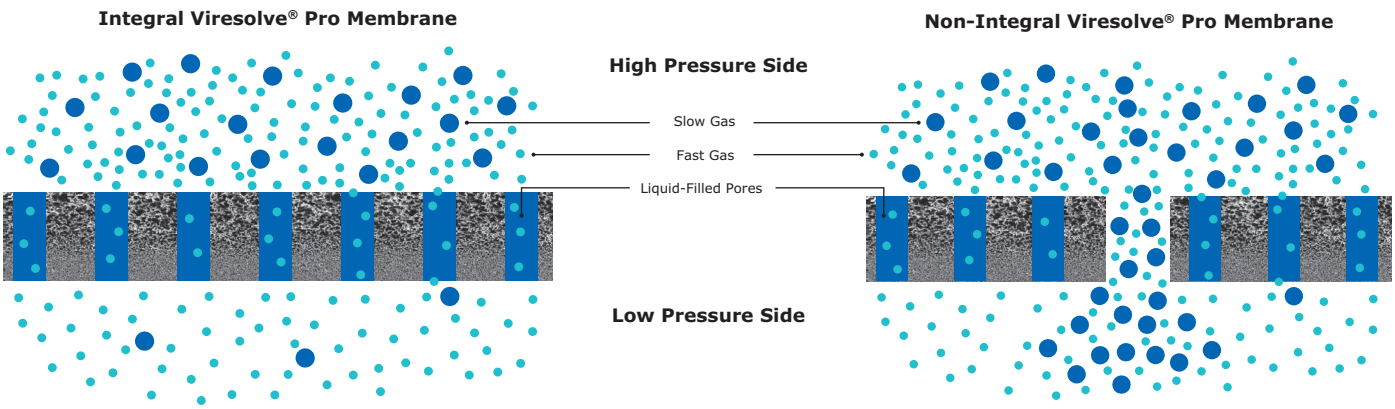




**Figure 3.**
Principles of Binary Gas Test. This proprietary test measures the composition of a mixture of two gases on the upstream and downstream sides of the Viresolve® Pro membrane to detect defects as small as 3-5 microns in size.

## High Capacity and Flux

The Viresolve® Pro Solution efficiently processes feed streams of different pH, conductivities and protein concentrations. When used upstream of Viresolve® Pro Devices, Viresolve® Pro Shield and Shield H enhance the throughput and processing robustness of filtration operations.

Figure 4 shows the results of throughput testing with Viresolve® Pro Devices alone (A) or in conjunction with Viresolve® Pro Shield and Shield H (B). In most cases, implementing the prefilter increased the capacity of the Viresolve® Pro Device by an average of two-fold. The Viresolve® Pro Solution enables rapid processing delivering mass flux in the 1.25-2.5 kg/m$^2$/hr range.

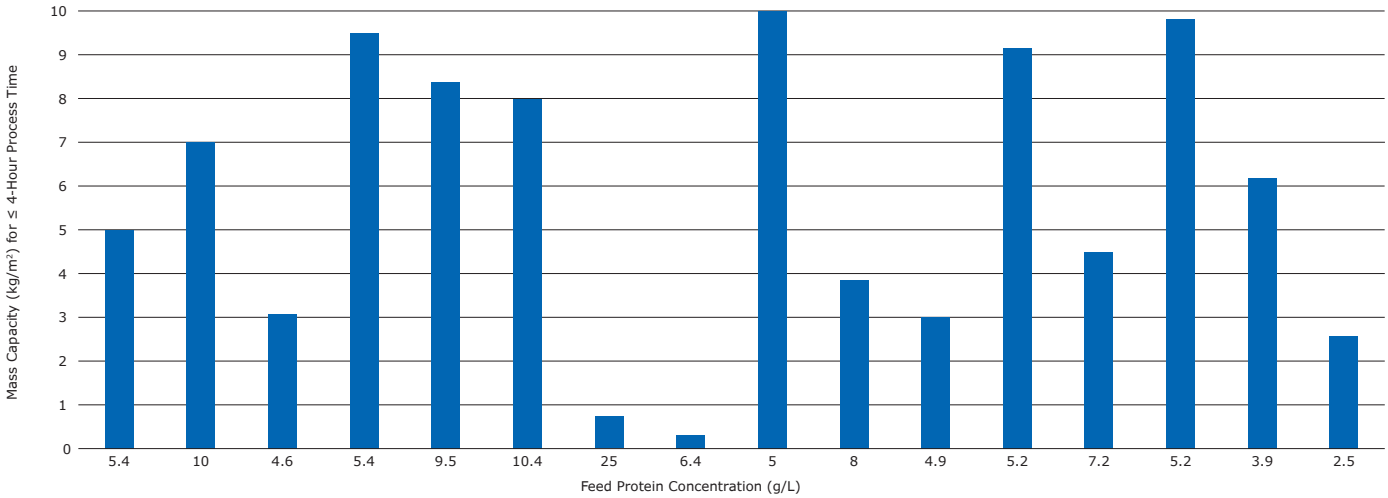



**Figure 4A.**
Mass capacity on Viresolve® Pro Devices with mAbs of different protein concentrations. In all cases, processing time was less than four hours.

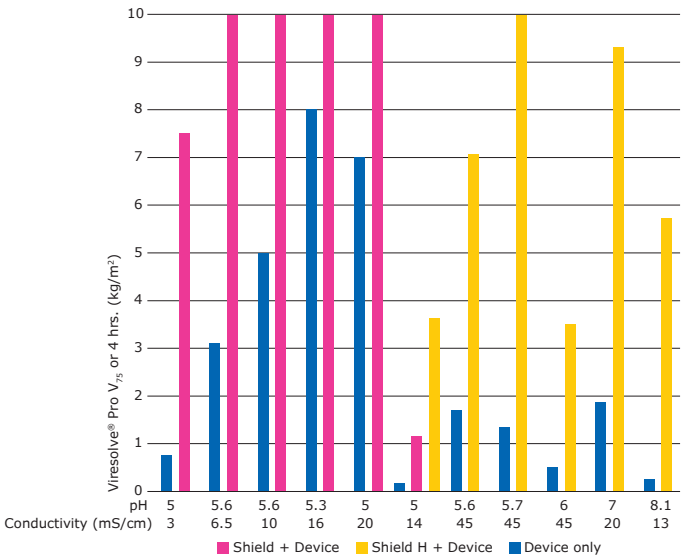



**Figure 4B.**
Mass capacity of the Viresolve® Pro Solution across a range of pH and conductivities. In all cases, processing was stopped at 75% flow decay or four hours.

## Flexible Manufacturing

The Viresolve® Pro Shield, Viresolve® Pro Shield H and Viresolve® Pro Devices are easily integrated into flexible, easy-to-use systems for pilot to full-scale manufacturing.

### Viresolve® Pro/Pro+ Magnus Holders for Viresolve® Pro Solution

Viresolve® Magnus Holders are designed for large volume processing. The Viresolve® Pro Magnus Holder is designed to run the Viresolve® Pro Device alone, while the Viresolve® Pro+ Magnus Holder is designed to run the Viresolve® Pro Device coupled with either the Viresolve® Pro Shield or Shield H.

- Quick and easy loading and unloading
- No product contact
- Minimized holder footprint with vertical orientation
- Rods in multiple lengths for various sized installations
- Simple manual hydraulics

### Mobius® FlexReady Solution for Virus Filtration

The Mobius® FlexReady Solution for large-scale virus filtration is an easy-to-use system featuring an optimized single-use flow path designed to fully support your virus filtration needs.

For more information on the Mobius® FlexReady Solution for large scale virus filtration, refer to datasheet DS1259EN00.

**Viresolve® Pro+ Magnus Holder**

**Viresolve® Pro Magnus Holder**

**Mobius® FlexReady Solution**

## Partner with a leader in viral safety

### Virus Filtration Process Development Service

Optimizing a virus filtration process involves evaluating the effects of multiple process parameters to identify conditions that will ensure a robust, consistent, and economical operation. We work side-by-side with development engineers and manufacturers, helping them develop efficient, cost-effective filtration operations.

We can help you:

- Maximize filtration efficiency
- Maximize process robustness
- Meet your economic targets

**The Viresolve® Pro Solution is supported by the Emprove® Program - your fast track through regulatory challenges.**

Complementing our product portfolio, the Emprove® Program provides three types of dossiers to support different stages of development and manufacturing operations such as qualification, risk assessment and process optimization. The dossiers consolidate comprehensive product-specific testing data, quality statements and regulatory information in a readily-available format to simplify your compliance needs.

### Viral Clearance Services

Viral clearance studies are critical to the validation of downstream processes, ensuring sufficient reduction of potential viral contaminants during downstream processing.

BioReliance® viral clearance studies are designed and executed by experts in regulatory, downstream processing, and virology at our facilities in Singapore, the U.S., and the UK. Our global experts can support you with your IND and BLA studies in accordance to regulatory guidelines for monoclonal antibodies, recombinant proteins, and plasma derivatives. Our dedicated project management support and local teams of experienced technical experts accelerate your time to results and minimize risk as you bring your product to market.

### Virus Filter Implementation Service

Our engineers can leverage the results of bench-scale studies to help implement your pilot or production scale virus filtration operation. This streamlines implementation and avoids the pitfalls that can impact production timelines and process economics.

## Nominal Dimensions and Weights

### Viresolve® Pro Shield, Viresolve® Pro Shield H, Viresolve® Pro Devices, and Holders

| Size | Nominal Dimensions | Nominal Effective Filtration Area | Nominal Weight |
|---|---|---|---|
| **Micro 40** | Height: 4.03 cm (1.59 in.)<br>Diameter: 4.37 cm (1.72 in.) | 3.4 $cm^2$ | Empty: 14.73 grams |
| **Modus 1.1** | Length: 18.62 cm (7.33 in.)<br>Width: 9.22 cm (3.63 in.)<br>Height: 5.92 cm (2.33 in.) | 0.017 $m^2$ | Empty: 0.37 Kg (0.8 lbs) |
| **Modus 1.2** | Length: 18.62 cm (7.33 in.)<br>Width: 9.22 cm (3.63 in.)<br>Height: 7.85 cm (3.09 in.) | 0.07 $m^2$ | Empty: 0.63 Kg (1.4 lbs) |
| **Modus 1.3** | Length: 18.62 cm (7.33 in.)<br>Width: 9.22 cm (3.63 in.)<br>Height: 13.56 cm (5.34 in.) | 0.22 $m^2$ | Empty: 1.39 Kg (3.1 lbs) |
| **Magnus 2.1** | Length: 34.30 cm (13.50 in.)<br>Width: 20.96 cm (8.25 in.)<br>Height: 4.42 cm (1.74 in.) | 0.51 $m^2$ | Empty: 2.6 Kg (5.7 lbs)<br>Full of water: 3.4 Kg (7.5 lbs) |
| **Magnus 2.2** | Length: 34.30 cm (13.50 in.)<br>Width: 20.96 cm (8.25 in.)<br>Height: 9.50 cm (3.74 in.) | 1.53 $m^2$ | Empty: 5.8 Kg (12.8 lbs)<br>Full of water: 8.3 Kg (18.3 lbs) |
| **Viresolve® Pro Magnus Holder (VPMH103000 / VPMH105000 / VPMH107000)** | Length: 78 cm (30.9 in.)<br>Width: 76 cm (30 in.)<br>Height: 127 cm (50 in.) | Not Applicable | 141.5 Kg (312 lbs) |
| **Viresolve® Pro+ Magnus Holder (VPMH203000 / VPMH205000 / VPMH207000)** | Length: 104 cm (40.9 in.)<br>Width: 76 cm (30 in.)<br>Height: 127 cm (50 in.) | Not Applicable | 186 Kg (410 lbs) |

## Materials of Construction

### Viresolve® Pro Shield, Viresolve® Pro Shield H, and Viresolve® Pro Devices, and Holders

| Device | Membrane | Components | O-Rings/Gaskets |
|---|---|---|---|
| **Micro 40** | Polyethersulfone (PES) | Bottom Endcap/Top Endcap: Polyvinylidene fluoride (PVDF) | Not Applicable |
| **Modus 1.1, Modus 1.2, Modus 1.3, Magnus 2.1, Magnus 2.2** | Polyethersulfone (PES) | Bottom Endcap/Top Endcap: Polyvinylidene fluoride (PVDF) | Silicone |

| Shield and Shield H | Membrane | Components | O-Rings/ Gaskets | Connections* |
|---|---|---|---|---|
| **Micro 40** | Polyethersulfone (PES) | Inlet Cap/Outlet Cap: Polypropylene/Polyethylene (copolymer) | Not Applicable | Inlet and Vent: Female Luer-Lok™ Fitting<br>Outlet: Male Luer Slip |
| **Modus 1.1, Modus 1.2, Modus 1.3** | Polyethersulfone (PES) | Bottom Endcap/Top Endcap: Polyvinylidene fluoride (PVDF) | Silicone | Inlet and Outlet: 1.91 cm (.75 in.) sanitary fittings<br>Integrated Vent: 0.32 cm (0.125 in.) with hose barb with double O-ring seal |
| **Magnus 2.1, Magnus 2.2** | Polyethersulfone (PES) | Bottom Endcap/Top Endcap: Polyvinylidene fluoride (PVDF) | Silicone | Inlet and Outlet: 3.81 cm (1.5 in.) sanitary fittings<br>Vent: 1.90 cm (0.75 in.) sanitary fitting for the port |

* Fittings sold seperately

## Materials of Construction (continued)

### Viresolve® Pro Shield, Viresolve® Pro Shield H and Viresolve® Pro Devices, and Holders

| Holders | Membrane | Components | O-Rings/ Gaskets | Connections |
|---|---|---|---|---|
| **Viresolve® Pro Magnus Holder** | Not Applicable | Plates & Frames: 316 L stainless steel | Not Applicable | Not Applicable |
| | | Clamp Rods: 300 series stainless steel | | |
| | | Fasteners, other components: 300 series stainless steel | | |
| **Viresolve® Pro + Magnus Holder** | Not Applicable | Plates & Frames: 316 L stainless steel | Not Applicable | Not Applicable |
| | | Clamp Rods: 300 series stainless steel | | |
| | | Fasteners, other components: 300 series stainless steel | | |
| **Fittings Kit** | Not Applicable | Polyvinylidene fluoride (PVDF) | Silicone | Not Applicable |

## Specifications

### Viresolve® Pro Shield, Viresolve® Pro Shield H and Viresolve® Pro Devices

| | |
|---|---|
| ISO® 9001 Quality Standard | These products are manufactured in a facility whose Quality Management System is approved by an accredited registering body to the appropriate ISO® 9001 Quality Systems Standard. |
| Particulate and Bioburden | These products are manufactured in an ISO® Class 8 (Per ISO® 14644-1) controlled environment for particulate classification only. |
| Animal Origin | All components used in the manufacturing of these products are either animal-free or in compliance with EMEA/410/01. |
| USP <87> Biological Reactivity Tests | Component materials for these products were tested and meet the criteria for non-cytotoxicity for the USP <87> Cytoxicity L929 MEM Elution Tests. |
| USP <88> Biological Reactivity Tests | Component materials for these products were tested and meet the criteria for USP <88> Biological Reactivity Tests for Class VI Plastics. |
| Bacterial Endotoxin | An aqueous extract from these products contains less than 0.25 EU/mL as determined by the Limulus Amebocyte Lysate (LAL) test. |
| Membrane Bacteriophage Retention | Membrane samples exhibited ≥ 4.0 LRV of φX 174 bacteriophage at a minimum challenge level of 107 pfu/cm$^2$ in the presence of a model protein at $V_{75}$. |
| Bacteriophage Retention | Viresolve® Pro Device samples exhibited ≥ 4.0 LRV of φX 174 bacteriophage at a minimum challenge level of $10^7$ pfu/cm$^2$ in the presence of a model protein at V75. |
| Non-Fiber Releasing | These products are non-fiber releasing filters as defined in 21 CFR 210.3(b)(6). |
| Hydraulic Stress Test | Samples were integral based on an Air/ Water Diffusion Test, before and after a forward stress to 4.1 bar (60 psid) at 25°C. |
| Manufacturing Integrity Test | All Viresolve® Pro Micro 40 Devices included in the Viresolve® Pro Micro 40 Device Kit must pass the Binary Gas Test.<br>All Viresolve® Pro Modus and Magnus Devices must pass the Pressure Hold, Water Flow Rate, Air/Water Diffusion Test and Binary Gas Test.<br>All Viresolve® Pro Shield and Viresolve® Pro Shield H must pass an aerosol particle challenge and housing pressure hold.<br>All Viresolve® Pro Devices exhibited an air diffusion flow rate at 3.4 bar (50 psig) in water at 25 °C of less than or equal to:<br>• 0.7 cc/min per Viresolve® Pro Modus 1.1 Device<br>• 2.7 cc/min per Viresolve® Pro Modus 1.2 Device<br>• 8.8 cc/min per Viresolve® Pro Modus 1.3 Device<br>• 20 cc/min per Viresolve® Pro Magnus 2.1 Device<br>• 60 cc/min per Viresolve® Pro Magnus 2.2 Device |
| Caustic Sanitization | These products may be sanitized by one 60-minute flush at 1.8 bar (25 psig) in 0.5 Normal Sodium Hydroxide at 25 °C followed by a maximum 16-hour static soak. |

## Ordering Information

| Description | Primary Use | Qty/Pk | Cat. No. |
|---|---|---|---|
| **Viresolve® Pro Device** | | | |
| Viresolve® Pro Micro 40 Device Kit | Process Development or Viral Clearance Evaluations | 9 | **VPMKVALNB9** |
| Viresolve® Pro Modus 1.1 Device | Small-scale studies/pilot | 1 | **VPMD101NB1** |
| Viresolve® Pro Modus 1.2 Device | Pilot/small-volume | 1 | **VPMD102NB1** |
| Viresolve® Pro Modus 1.3 Device | Pilot/small-volume | 1 | **VPMD103NB1** |
| Viresolve® Pro Magnus 2.1 Device | Large-volume processing | 1 | **VPMG201NB1** |
| Viresolve® Pro Magnus 2.2 Device | Large-volume processing | 1 | **VPMG202NB1** |
| **Viresolve® Pro Shield** | | | |
| Viresolve® Pro Micro Shield Kit | Process Development or Viral Clearance Evaluations | 9 | **VPMSKITNB9** |
| Viresolve® Pro Modus 1.1 Shield | Small-scale studies/pilot | 1 | **VPPS101NB1** |
| Viresolve® Pro Modus 1.2 Shield | Pilot/small-volume | 1 | **VPPS102NB1** |
| Viresolve® Pro Modus 1.3 Shield | Pilot/small-volume | 1 | **VPPS103NB1** |
| Viresolve® Pro Magnus 2.1 Shield | Large-volume processing | 1 | **VPPS201NB1** |
| Viresolve® Pro Magnus 2.2 Shield | Large-volume processing | 1 | **VPPS202NB1** |
| **Viresolve® Pro Shield H** | | | |
| Viresolve® Pro Micro Shield H Kit | Process Development or Virus Validation Studies | 9 | **VPMHKITNB9** |
| Viresolve® Pro Modus 1.1 Shield H | Small-scale studies/pilot | 1 | **VPPH101NB1** |
| Viresolve® Pro Modus 1.2 Shield H | Pilot/small-volume | 1 | **VPPH102NB1** |
| Viresolve® Pro Modus 1.3 Shield H | Pilot/small-volume | 1 | **VPPH103NB1** |
| Viresolve® Pro Magnus 2.1 Shield H | Large-volume processing | 1 | **VPPH201NB1** |
| Viresolve® Pro Magnus 2.2 Shield H | Large-volume processing | 1 | **VPPH202NB1** |
| **Holders, Accessories, Services and Spare Parts** | | | |
| Viresolve® Pro Magnus Holder | For 1 to 3 Viresolve® Pro Devices | 1 | **VPMH103000** |
| | For 1 to 5 Viresolve® Pro Devices | 1 | **VPMH105000** |
| | For 1 to 7 Viresolve® Pro Devices | 1 | **VPMH107000** |
| Viresolve® Pro+ Magnus Holder | For 1 to 3 Viresolve® Pro Devices and 1 to 3 Viresolve® Pro Shields or Viresolve® Pro Shield H | 1 | **VPMH203000** |
| | For 1 to 5 Viresolve® Pro Devices and 1 to 5 Viresolve® Pro Shields or Viresolve® Pro Shield H | 1 | **VPMH205000** |
| | For 1 to 7 Viresolve® Pro Devices and 1 to 7 Viresolve® Pro Shields or Viresolve® Pro Shield H | 1 | **VPMH207000** |
| Split clamp insert | | 1 | **VPMHINSERT** |
| Rod handle | | 1 | **VPMHRDKN0B** |
| Rods | For 1 to 3 Viresolve® Pro Devices | 2 | **VPMHRD0103** |
| | For 1 to 5 Viresolve® Pro Devices | 2 | **VPMHRD0105** |
| | For 1 to 7 Viresolve® Pro Devices | 2 | **VPMHRD0107** |
| Fittings Kit (three 3.81 cm (1.5 in.) sanitary fittings, two 3.81 cm (1.5 in.) blanks, one 1.27 cm (.5 in.) vent fitting and one 1.27 cm (.5 in.) blank) | Viresolve® Pro and Pro+ Magnus Holder | 1 | **VPMHADAPSK** |
| 3.81 cm (1.5 in.) Sanitary fittings | For feed/permeate port | 6 | **VPMHADAPSF** |
| 3.81 cm (1.5 in.) Blanks | For feed/permeate port | 6 | **VPMHADAPSB** |
| 1.27 cm (.5 in.) Vent fittings | For vent port | 6 | **VPMHADAPVF** |
| 1.27 cm (.5 in.) Vent blanks | For vent | 6 | **VPMHADAPVB** |
| Hydraulic pump | | 1 | **MP0DHYPUMP** |
| Pressure gauge | | 1 | **MP0DHYGAGE** |
| Hydraulic fluid (1 liter) | | 1 | **MP0DHFLUID** |

## Ordering Information (continued)

| Description | Primary Use | Qty/Pk | Cat. No. |
|---|---|---|---|
| **Viresolve® Pro and Pro+ Magnus Holder Services** | | | |
| IQ/OQ Protocol | Commissioning, installation and operational qualification (IQ/OQ) protocol | 1 | **DOCVMHIQOQ** |
| IQ/OQ Protocol and Service | Commissioning, installation and operational qualification (IQ/OQ) protocol and service | 1 | **SSVIOQMGN** |
| On-Site Preventive Maintenance (PM) Service | Equipment checks, testing for proper functionality, adjustments and parts replacement (sold separately). | 1 | **CSVOPMMGN** |
| **Mobius® FlexReady Solutions for Virus Filtration** | | | |
| Refer to data sheets (DS2562EN00 and DS1259EN00) for specific ordering information. | | | |

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

## To place an order or receive technical assistance

Please visit **EMDMillipore.com/contactPS**

For additional information, please visit **EMDMillipore.com**



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31916
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Millipore®
Filtration, Separation
& Preparation

**Specification Sheet**

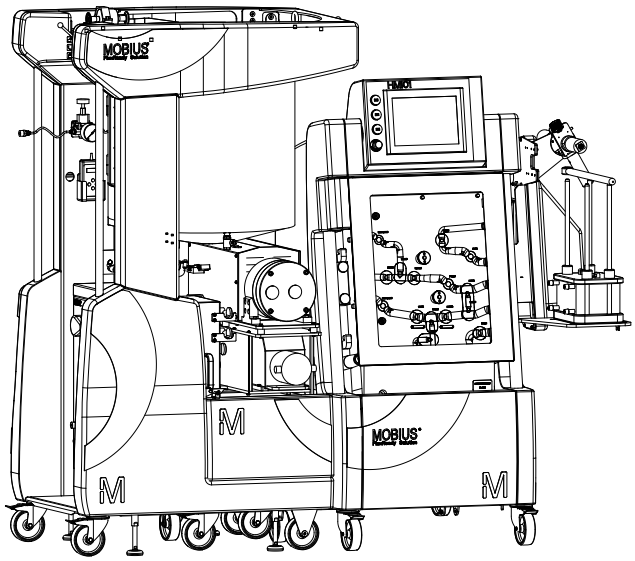
# Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF

## Features

- Flexible design options
- Unique feed tank design and performance
- Smart Flexware® full single-use flow paths
- Excellent product recovery and purification
- Implement with ease and lower risks
- Full process automation with flexible recipes using the Common Control Platform® (CCP®)

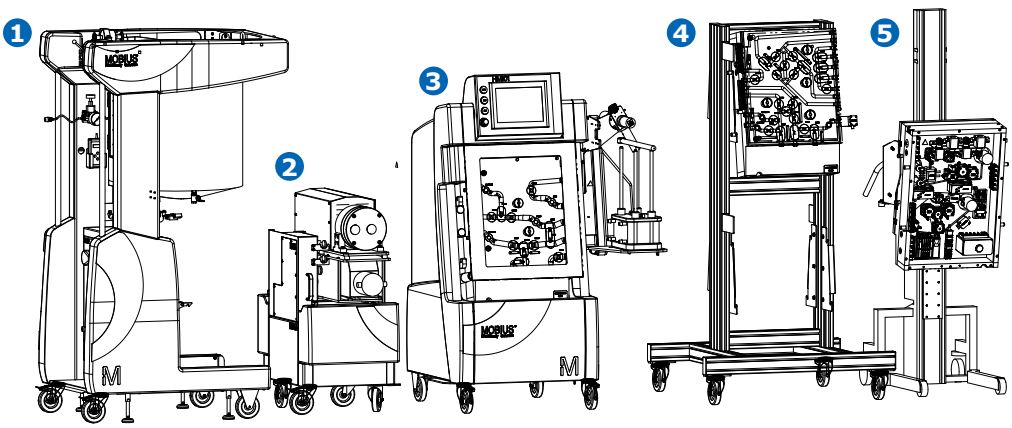
## Benefits

- Ability to produce higher final concentrations
- Consistently deliver safety and quality with less cleaning validation and lower risk of cross contamination
- Ability to meet unique requirements
- Agile system able to perform at multiple scales for both TFF and Chromatography
- Simplified training requirements




**TF2S:** 18L/min – 0.5 to 5.0 m² Pellicon® cassettes or similar

**TF3S:** 40L/min – 2.0 to 10.0 m² Pellicon® cassettes or similar




1. Tank cart
2. Pump and manifold cart
3. Smart cart with exchangeable clamshell
4. Clamshell storage rack
5. Chamshell lift



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# General Specifications

## System Dimensions

| | | |
|---|---|---|
| Carts connected H x L x W in. (mm) | TF2S | 2022 x 2160 x 974 +/- 20 mm |
| | TF3S | 2022 x 2160 x 1014 +/- 20 mm |

## Net Weight

| | | |
|---|---|---|
| Tank cart 50 L | | 280 kg |
| Tank cart 100 L | | 310 kg |
| Tank cart 200 L | | 330 kg |
| Smart cart with clamshell | | 430 kg |
| Pump cart with 2 pumps and manifold | TF2S | 200 kg |
| | TF3S | 260 kg |

## Environmental Operating Conditions

| | |
|---|---|
| Product temperature range | 2 to 45 °C |
| System operation temperature | 2 to 30 °C (20 to 30 °C for TF3S) |
| Operating humidity | 10%–90% (non-condensing) |
| Operating pressure | 4 bar max: feed / retentate assemblies<br>2 bar max: transfer / filtrate assemblies<br>Atmospheric pressure: feed bag |

# Fluidic Sections

## Feed Section

| | | |
|---|---|---|
| Feed container volume | TF2S | 50 L/100 L/200 L in LLDPE or stainless steel container with jacket |
| | TF3S | 200 L in LLDPE or stainless steel container with jacket |
| Tank jacket volume | | 50 L tank 3.3 L<br>100 L tank 6.0 L<br>200 L tank 7.8 L |
| Pump model | TF2S | Quattroflow 1200 SU |
| | TF3S | Quattroflow 4400 SU with 3° shaft |
| Flow at 4 bar max | TF2S | 2 to 20 L/min |
| | TF3S | 4 to 40 L/min |
| Minimal working volume (w/o cassettes feed volume) | TF2S | 0,7 L -1,8 L from 2 to 18 L/min* |
| | TF3S | 2,2 L -4,0 L from 4 to 40 L/min* |
| Maximum viscosity | | 35cP |
| Pressure sensor | Non-intrusive | 0-4 bar +/-0.2 bar; Security switch set at 4.4 bar |
| Tank weight | | 0.3% FS |
| Control on feed pump | | Fixed position (speed in %) or flow control or pressure drop control |
| Precision of calculated feed flow | | +/-10% with feed pressure at least 1 bar |
| Temperature sensor feed container | | 2-45 °C +/-2 °C |

*May vary slightly depending on the configuration system and other parameters.

### Transfer Section

| | | |
|---|---|---|
| Pump model | | Quattroflow™ 1200 SU |
| Flow at 2 bar | TF2S<br>TF3S | 5m²: 2 to 9 L/min<br>10m²: 2 to 20 L/min |
| Transfer pump control | | Fixed position (speed in %) or level control |
| Pressure sensor | | 0-4 bar +/-0.2 bar; Security switch set at 2.3 bar |

### Retentate Section

| | | |
|---|---|---|
| Pressure sensor | Non-intrusive | 0-4 bar +/-0.2bar |
| Sampling | | Optional zero dead leg device – NovaSeptum® sampling solution compatible |
| Volume factor concentration | VCF | Precision better than 2% |
| Retentate PCV control | | Fixed position (% of closure) or TMP control or retentate pressure control |

### Filtrate Section

| | | |
|---|---|---|
| Pressure sensor | Non-intrusive | 0-4 bar +/-0.2bar |
| Conductivity and UV | In-line Single-use Cell (Optek) and Multi-use | Conductivity: 0 to 100 µS/cm +2% FS<br>UV: 0 to 2 AU +2% FS<br>OPL: 10 mm<br>Wavelength: 280 nm / 300 nm |
| Weight with Mobius® weight scale | TF2S | 0 to 600 Kg + 0.3 % FS |
| Weight with user supplied weight scale | TF2S<br>TF3S | 0 to 1000 Kg (accuracy linked to component connected)<br>0 to 1500 Kg (accuracy linked to component connected) |
| Flowmeter | Non intrusive (ultrasonic by Emtec) | From 2LPM: +/-5% MV<br>Below 2LMP: +/-0,12 LPM |
| Sampling | | Optional zero dead leg device<br>NovaSeptum® sampling solution compatible |
| Filtre PCV control | | Fixed position (% of opening) or TMP |

**Important Note:**

The single-use cell (SUC) is factory calibrated by Optek and for best performance the end-user should enter in the C8000 transmitter, the K factor delivered with each new sensor assembly.

As calibration is dependent on each SUC installed, the calibration loop should be verified by the end-user prior and/or after each batch (as per customer internal standard operating procedures.)

In consequence, during internal release test, only the proper wiring of the loops is tested using:
- Calibrated UV filters
- A conductivity tester supplied by Optek to simulate a conductivity value

## Documentation

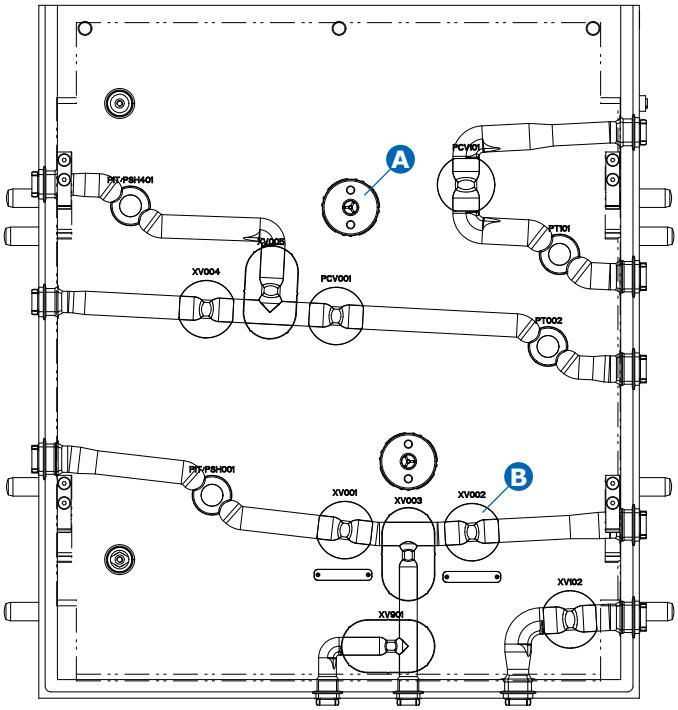
User guide on CD

Access to template recipes upon request

1. FDA Guidance for Industry Process Validation: General Principles and Practices – January 2011
2. Annex 15 to the EU Guide to Good Manufacturing Practice Qualification and Validation – July 2001
3. Mobius® FlexReady solution with Smart Flexware Assemblies for tangential – Flow Filtration Performance Guide – AN4465EN00

## Smart Flowpath

The smart flowpath is a unique and patented fluid management device featuring fewer connections than other designs. Fewer connections provide improved process effectiveness through reduced working volume and protein shearing, reduced leak risks, and maximum product recovery.




**Feed**

XV001, XV002: Normally Closed Valves
PIT/PSH001 : High Pressure Switch Sensor

**Retentate**

PCV001: Normally Opened Control Valve
XV004: Normally Opened Valve
PT002: Retentate Pressure Sensor

**Filtrate**

PCV101: Normally Closed Control Valve
PT101: Filtrate Pressure Sensor

**Transfer**

XV005: Normally Closed Valve
PIT/PSH401: High Pressure Switch Sensor

**Drain/recovery**

XV901, XV003: Normally Closed Valves

**Filtrate drain**

XV102: Normally Closed Valve

**A: Internal door locks and sensors**

**B: Silicone Valve Pads**

### Endurance

| | |
|---|---|
| Active process time | 12 h at 50 cycles per valve |
| Valve pads longevity | 2500 cycles / 6 months |

### Wetted Materials

| | |
|---|---|
| Tubing | Silicone |
| Smart Flexware® Assembly | Pureflex™ film and PE fittings |
| Feed Bag Assembly | Pureflex™ film |
| Pump Head | EPDM, Santoprene® and Polypropylene |
| Liners | Polysulfone |
| Connectors | Polypropylene and Polysulfone |
| Multi-use UV & Conductivity Sensors | Quartz, EPDM and stainless steel 316 L |
| Single Use UV & Conductivity Sensors | Polysulfone Quartz, EPDM and stainless steel 316 L (pins only) |
| Retentate Low Dead Volume TC Sampler | DMDA-1250 NT 7, sampler: HDPE O-ring: silicone |
| TFF Mixer Vortex Breaker & Diverter Plate | HDPE |

### Regulatory and Quality Compliance

| | |
|---|---|
| USP <87> Biological reactivity *in vitro*, USP <88> Biological Reactivity *in vivo*, Plastic Calss VI | Compliant |
| Irradiation dose | 25–40 kG |
| 21 CFR Part 11 | Compliant ready |
| 2004/108/CE Electromagnetic Compatibility (EMC) | Compliant |
| 2006/42/CE Machinery Directive | Compliant |
| PSE | Exempt |
| ASME U-1 code | Tank Jacket is compliant |

## Data and System Control

| | |
|---|---|
| PLC | Allen-Bradley CompactLogix™ |
| Control software system | Microsoft® Windows® 7 |
| Operator interface panel type | iFix® |
| Operator interface | 12.1” tiltable touch screen |
| Languages | English, German, Spanish, Japanese, French, Italian, Chinese, Korean |
| Security | Based on Microsoft® Windows® operating system<br>Configurable user access group levels (4 pre-configured) |
| Data acquisition | Stored in Read Only Database |
| PC | B&R |
| Data | 21 CFR Part 11 compliance ready |
| Manufacturing | GMP compliance ready |

## Utilities Connections

| | |
|---|---|
| OPC server | RJ45 |
| Keyboard | USB |
| External storage media | USB |
| Mouse | USB |
| Air | 6–10 bar oil free at 4 L/min max |

### Power Supply

| | |
|---|---|
| Smart Cart | 220–240 VAC, 50/60 Hz, 1 phase, 3.9 A<br>Or 100-120 VAC, 50/60 Hz, 1 phase, 8.4 A<br>Maximum power consumption 1 kW |
| Tank Cart | 220–240 VAC, 50/60 Hz, 1 phase, 0.4 A (50 L) – 1.8 A (100/200 L)<br>Or 100–120 VAC, 50/60 Hz, 1 phase, 0.8 A (50 L) – 3.7 A (100/200 L)<br>Maximum power consumption 0.1 kW (50L) to 0.4 kW (100/200L) |
| Pump cart (TF3S) | 3 x 200–240 VAC, 50/60 Hz, 3 phases + neutral + ground, 10 A<br>Or 3 x 400–460 VAC, 50/60 Hz, 3 phases + neutral + ground, 5 A<br>Maximum power consumption 3 kW |

Millipore®
Preparation, Separation,
Filtration & Testing Products

Data Sheet

# Pellicon® 3 Cassettes with Ultracel® Membrane

## The optimum tangential flow filtration devices for monoclonal antibodies and other therapeutic proteins.

Pellicon® 3 cassettes with Ultracel® membrane are advanced, high-performance cassettes that are ideal for today's higher titer therapeutic antibodies, as well as for the more demanding filtration processes that require higher operating pressures, temperatures, concentrations and caustic cleaning regimes.

From small-scale to full-scale production, Pellicon® 3 cassettes are designed for use in research, process scale-up/scale-down, applications development and full-scale manufacturing. The Pellicon® 3 cassette design and automated manufacturing process provides unbeatable performance consistency and enhanced linear scalability between cassette sizes. Pellicon® 3 cassettes also offer greater cassette size selection for improved scale-up and scale-down process development. The streamlined design allows operators to quickly and easily handle, install and remove Pellicon® 3 cassettes. The materials of construction are compatible with a broad range of chemical cleaning agents that many TFF systems require to ensure proper sanitization.

### Benefits

- Optimum product recovery using proven composite membrane technology
- Fast, reliable scale-up/-down from lab to production scale
- Rugged, reliable design ideally suited to filtration processes with higher operating pressures, temperatures and caustic cleaning regimes
- Automated manufacturing delivers unbeatable performance consistency and reliability
- Easy to install and clean
- Extreme temperature and chemical compatibility
- Choice of screens to best optimize your process

### Applications

- Monoclonal antibodies
- Recombinant and non-recombinant proteins
- Vaccine



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# Specifications

## Materials and Assembly

| | |
|---|---|
| **Materials of Construction** | • Polypropylene<br>• Polyethylene<br>• Composite regenerated cellulose<br>• Thermoplastic elastomer<br>• Stainless steel (0.57 $m^2$ and 1.14 $m^2$ cassettes only) |
| **Storage Solution** | 3-4% benzyl alcohol, 20% glycerin and water |
| **Membrane** | Ultracel® membrane – Composite regenerated cellulose (regenerated cellulose membrane cast on a microporous polyethylene membrane) |
| **Assembly Design** | Automated assembly and testing of heat-sealed packets bound together by an injection-molded polypropylene jacket |

## Maximum Operating Conditions

| | |
|---|---|
| **Recommended Feed Flow Rate** | 4–8 $L/m^2/min$ |
| **Inlet Pressure** | 100 psi |
| **Forward Transmembrane Pressure** | 80 psi (5.5 bar) at 4–40°C, 200 hours continuous<br>40 psi (2.7 bar) at 4–50°C, 50 hours continuous |
| **Reverse Transmembrane Pressure** | 30 psi (2.1 bar) at 25°C, 3 min intervals, 10 cycles |
| **Maximum Caustic Exposure (One Time)** | 0.5N NaOH at 50°C up to 50 hours |
| **Operating pH Range** | 2–13 |

## Regulatory Information

| | |
|---|---|
| **Component Material Toxicity** | Component materials were tested and meet the criteria of the USP <88> Biological Reactivity Tests for Class VI Plastics. |
| **Good Manufacturing Practices** | These products are manufactured in a facility that adheres to current Good Manufacturing Practices. |
| **ISO® 9001 Quality Standard** | This product was manufactured in a facility whose Quality Management System is approved by an accredited registering body to the appropriate ISO® 9001 Quality Systems Standard. |
| **100% Integrity Tested in Manufacturing** | Each unit must pass our integrity test based on air flow through the fully wetted membranes of the filter. |
| **Validated Production Process** | This product was fabricated using a validated manufacturing process. Principles of statistical process control and determinations of process capability have been applied to critical variables in the device fabrication process. In-process controls are used to assure stability of the process. |

## Nominal Dimensions

| Filtration Area | Length mm (in.) | Width mm (in.) | Thickness mm (in.) |
|---|---|---|---|
| **C-Screen** | | | |
| **88 $cm^2$** | 206 (8.1) | 56 (2.2) | 8.3 (0.33) |
| **0.11 $m^2$** | 206 (8.1) | 56 (2.2) | 24 (0.93) |
| **0.57 $m^2$** | 206 (8.1) | 178 (7.0) | 26 (1.03) |
| **1.14 $m^2$** | 206 (8.1) | 178 (7.0) | 42 (1.66) |
| **D-Screen** | | | |
| **88 $cm^2$** | 206 (8.1) | 56 (2.2) | 8.3 (0.33) |
| **0.11 $m^2$** | 206 (8.1) | 56 (2.2) | 25 (0.98) |
| **0.57 $m^2$** | 206 (8.1) | 178 (7.0) | 29 (1.13) |
| **1.14 $m^2$** | 206 (8.1) | 178 (7.0) | 45 (1.78) |

## Hold-Up Volumes

| Membrane Area | Pellicon® 3 Cassettes with Ultracel® membrane with C Screen | | Pellicon® 3 Cassettes with Ultracel® membrane with D Screen | |
|---|---|---|---|---|
| | Feed Channel (mL) | Permeate Channel (mL) | Feed Channel (mL) | Permeate Channel (mL) |
| **88 cm²** | 1.5 | 2.4 | 3.6 | 2.0 |
| **0.11 m²** | 18 | 15 | 23 | 17 |
| **0.57 m²** | 85 | 68 | 118 | 75 |
| **1.14 m²** | 170 | 127 | 227 | 138 |

# Ordering Information

## Pellicon® 3 Cassettes with Ultracel® Membrane

| Description | Cat. No. |
|---|---|
| **3kD NMWL with C-Screen** | |
| 88 cm² | **P3C003C00** |
| 0.11 m² | **P3C003C01** |
| 0.57 m² | **P3C003C05** |
| 1.14 m² | **P3C003C10** |
| **5kD NMWL with C-Screen** | |
| 88 cm² | **P3C005C00** |
| 0.11 m² | **P3C005C01** |
| 0.57 m² | **P3C005C05** |
| 1.14 m² | **P3C005C10** |
| **10kD NMWL with C-Screen** | |
| 88 cm² | **P3C010C00** |
| 0.11 m² | **P3C010C01** |
| 0.57 m² | **P3C010C05** |
| 1.14 m² | **P3C010C10** |
| **30kD NMWL with C-Screen** | |
| 88 cm² | **P3C030C00** |
| 0.11 m² | **P3C030C01** |
| 0.57 m² | **P3C030C05** |
| 1.14 m² | **P3C030C10** |
| **30kD NMWL with D-Screen** | |
| 88 cm² | **P3C030D00** |
| 0.11 m² | **P3C030D01** |
| 0.57 m² | **P3C030D05** |
| 1.14 m² | **P3C030D10** |

## Accessories

| Holder Type | Cassette Size | Area Range | Cat. No. |
|---|---|---|---|
| **Pellicon® 3 Cassette Holders** | | | |
| Stainless Steel Mini-Holder | 88 cm² and 0.11 m² | 88 cm² to 0.55 m² | **XX42PMINI** |
| Acrylic Cassette Holder Low Retentate Volume | 0.57 m² and 1.14 m² | 0.57 m² to 5.7 m² | **XX42PRV60** |
| Stainless Steel Holder | 0.57 m² and 1.14 m² | 0.57 m² to 5.7 m² | **XX42P0080** |
| Stainless Steel Cassette Holder and Assembly | 0.57 m² and 1.14 m² | 0.57 m² to 5.7 m² | **XX42P0K80** |
| Manifold Support Plate | 0.57 m² and 1.14 m² | NA | **XXPEL3MAP** |
| Process Scale Holder | 0.57 m² and 1.14 m² | 1.14 m² and up | **Contact Local Representative** |
| Hydraulic Process Scale Holder | 0.57 m² and 1.14 m² | 1.14 m² and up | **Contact Local Representative** |

## Cleaning

| Description | Cat. No. |
|---|---|
| Sodium hydroxide solution 0.5 mol/L suitable for biopharmaceutical production EMPROVE® bio | **137060** |
| Sodium hydroxide solution 1 mol/L suitable for biopharmaceutical production EMPROVE® bio | **137031** |
| Sodium hydroxide solution 25% low iron suitable for biopharmaceutical production EMPROVE® bio | **480659** |

## Single-Pass TFF Accessories

| Description | Cat. No. |
|---|---|
| Diverter plate and silicone gasket kit for 88 cm² cassette | **XXSPTFF01** |
| Diverter plate for 0.57 m² and 1.14 m² cassettes | **XXSPTFF02** |
| Retentate collection plate for 0.57 m² and 1.14 m² cassettes | **XXSPTFF03** |

Application Note

# A Hands-On Guide to Ultrafiltration/ Diafiltration Optimization using Pellicon® Cassettes

In ultrafiltration (UF) tangential flow filtration (TFF) systems, operating parameter selection will have far reaching impact as the process is scaled to full-scale manufacturing levels. While there are many factors that contribute to final system design, several key parameters should be optimized early in the process development phase. The goal is to develop a robust process with the following success criteria: superior product quality, consistent and high product yield, reproducible process flux and time, and a cleaning regime that allows extended membrane reuse.

The following basic experiments should be considered during development of processing methodology:

- Optimization
  - Impact of transmembrane pressure (TMP) and feed flow on process flux and retention
  - Impact of product concentration and buffer conditions on process flux and retention
  - Impact of diavolumes on buffer exchange and contaminant removal
- Paper design and full process simulation with chosen processing parameters

Typically, the first three experiments are performed sequentially to bracket process performance and obtain data for analysis. This information is then combined with actual manufacturing considerations (batch volume, process time, etc.) to design a process simulation.

The purpose of a process simulation is to duplicate the entire manufacturing process in a scale-down format, to confirm sizing, and to assess preliminary product quality and yield. The intent is to develop an optimized process, on the bench, that will efficiently scale-up to meet full-scale manufacturing expectations.

| Sequence | Purpose |
|---|---|
| 1. TMP Excursion at Initial Concentration ($C_{b\ initial}$) | • Determine TMP for UF/DF<br>• Determine Feed Flow ($Q_F$) for UF/DF<br>• Demonstrate Flux Stability<br>• Confirm Retention of Product |
| ↓ | |
| 2. Concentration / Volume Reduction ($C_{b\ initial} \rightarrow C_{b\ final}$) | • Determine Flux as Function of Concentration<br>• Determine Placement of Diafiltration Step<br>• Determine Flux as Function of Buffer Conditions |
| ↓ | |
| 3. TMP Excursion at Final Concentration ($C_{b\ final}$) | • Determine TMP for UF/DF<br>• Determine Feed Flow ($Q_F$) for UF/DF<br>• Confirm Retention of Product |
| ↓ | |
| 4. Diafiltration / Buffer Exchange | • Determine Diavolume Requirement<br>• Confirm Retention of Product during DF |
| ↓ | |
| 5. Product Recovery | • Crude Assessment of Step Yield<br>• Product Quality Evaluation |

Figure 1. Basic Optimization Experiments

**Use this step-by-step guide to develop a robust UF/DF process with Pellicon® cassettes (cutoffs of 100 kD and lower) that will deliver superior product quality, reproducible results, and high yields.**

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

The following are step-by-step protocols for basic optimization experiments.

## Set-up and Installation Procedure

Refer to the *Maintenance Procedures for Pellicon® and Pellicon® 2 Cassette Filters (P17512)* or the *Pellicon® 3 Filters Installation and User Guide (AN1065EN00)* when performing actual set-up and installation of Pellicon® cassettes.

1. Assemble the TFF system as shown in Figure 2.
2. Install the Pellicon® cassette(s) (Pellicon® 2 Mini with 0.1 $m^2$ membrane area, Pellicon® 3 with 0.11 $m^2$ membrane area) in the appropriate Pellicon® holder.
3. Flush the system with water, clean with the appropriate cleaning agent (per appropriate maintenance guide), and flush again.

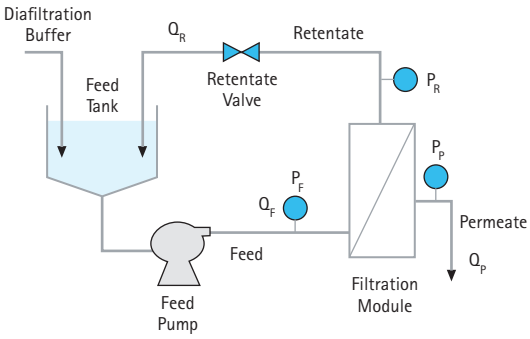



**Figure 2.** Schematic of a TFF System

## Equilibration Procedure

1. Add 3 L/$m^2$ of the appropriate buffer to the feed tank. *Example:* 0.1 $m^2$ membrane area x 3 L/$m^2$ = 0.3 L buffer
2. Direct the retentate and permeate to a waste container.
3. Start the feed pump and achieve the following conditions by partially closing the retentate valve and adjusting the pump speed:
   - Feed flow of 5 L/min/$m^2$
   - Retentate pressure of 2 – 15 psi (0.14 – 1.03 bar) to achieve approximately 30% conversion
4. When half the buffer has been flushed, put the system in total recycle mode[1] and recirculate for 10 minutes; verify that the pH and conductivity in the system have been equilibrated to the level of the starting buffer.
5. Direct the retentate and permeate to a waste container.
6. When the feed tank level reaches the minimum level, open the retentate valve fully and stop the feed pump to prevent the introduction of air into the system.

## Part 1. TMP Excursion at Initial Concentration

1. Add sufficient volume of product to the feed reservoir such that final volume or concentration target can be reached or slightly exceeded (approximately 1 – 1.5 L of final product at final concentration per $m^2$). *Example:* if $C_{initial}$ = 10 g/L and $C_{retentate}$ = 80 g/L, then the concentration factor is 8X. If the minimum achievable final volume for 0.1 $m^2$ is 0.1 L, calculate the required initial volume:

   $$V_{initial} = V_{minimum} \times VCF = 0.1\ L \times 8X = 0.8\ L$$

2. Open the retentate valve fully and configure system in total recycle mode.
3. Start the feed pump and achieve the following conditions by partially closing the retentate valve and adjusting the pump speed:
   - Recommended feed flow ($Q_F$) rate for the membrane device, typically 5 L/min/$m^2$ for Pellicon® 2 and 3 cassettes
   - Minimal TMP, typically 2 – 5 psi (0.14 – 0.34 bar) for more open membranes and 10 psi (0.69 bar) for tighter membranes.
4. Recirculate the product for 10 – 15 minutes and ensure that stable process flux is achieved[2].
5. Record temperatures, pressures, and flows; sample feed and permeate for product retention[3].
6. Increase TMP by 5 – 10 psid (0.34 – 0.69 bar) by manipulating the retentate valve while keeping the feed flow constant. For more open membranes increase by 2 – 5 psid (0.14 – 0.34 bar). Repeat steps 4 and 5.
7. Repeat step 6 until flux begins to level off[4]; typically 4 – 6 TMP values are evaluated in total.
8. Open the retentate valve fully and allow system to continue in a total recycle.
9. Increase or decrease the feed flow by 2 – 3 L/min/$m^2$ and repeat steps 4 through 8. If desired, a third feed flow rate can be investigated.
10. Plot the data as shown in Figure 3.

| | |
|---|---|
| Tight membranes (1 kD, 5 kD, etc.) | Can use large TMP increases since optimum is typically > 30 psi |
| Open membranes (50 kD, 100 kD, etc.) | Can use small TMP increases since optimum is typically < 10 psi |

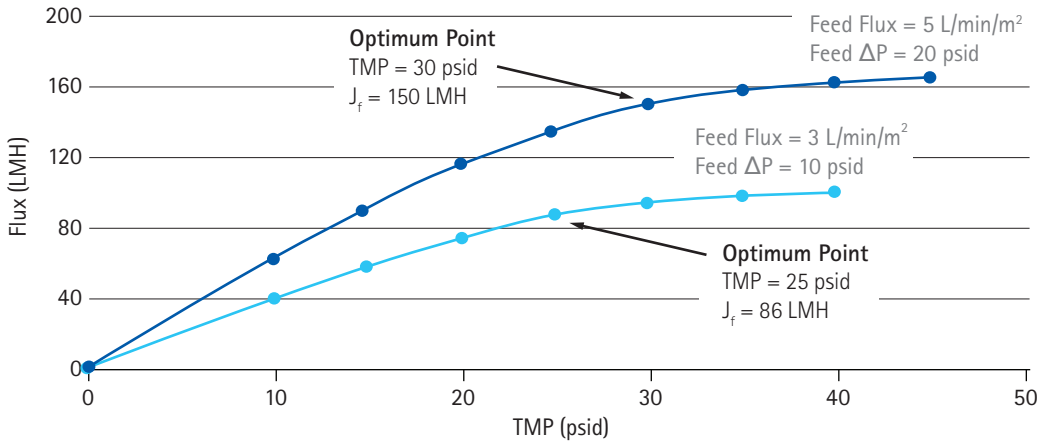



**Figure 3.**
TMP Excursion at Two Feed Flows

| | $Q_1$ = 5 L/min/m² | $Q_2$ = 3 L/min/m² | $Q_1/Q_2$ |
|---|---|---|---|
| A [m²] | Volume / Time / 150 LMH | Volume / Time / 86 LMH | 0.57 |
| $Q_F$ [L/min] | (5 L/min/m²) x Volume / Time / 150 LMH | (3 L/min/m²) x Volume / Time / 86 LMH | 0.95 |

**Table 1.** Membrane Area vs. Pump Feed Rate (Figure 3)

## Calculations

The appropriate combination of feed flow rate and TMP will maximize flux while minimizing the impact of pumping and shear on the product. The appropriate combination of these two parameters will also minimize processing time and/or membrane area. To calculate the optimum feed flow, compare the required membrane area with the required pump rate at each of the two feed flow conditions, as shown in Table 1.

Membrane Area [m²] =
Process Volume [L] / (Flux [LMH] x Process Time [h])

*In Figure 3:*

$Area_{Q1} = 0.57 \times Area_{Q2}$

Pump feed rate [L/min] =
Feed flux [L/min/m²] x Area [m²]

*In Figure 3:*

$Pump\ feed\ rate_{Q1} = 0.95 \times Pump\ feed\ rate_{Q2}$

In this example it is advantageous to run at the higher feed flow, $Q_1$, since it only requires 57% of the membrane area used at the lower feed flow rate at almost the identical pump feed rate.

### Note:

- Anticipated final volume of over-concentrated product must exceed minimum working volume of membrane system at selected feed flow rate ($Q_F$); avoid introduction of air and maintain uniform mixing at end of volume reduction.
- Move from least to greatest fouling conditions:
  - Do not test into pressure-independent regime (past the knee of the flux vs. TMP curve)[4]
  - Avoid exceeding 30 – 40% conversion ratios
- Check hysteresis if possible by returning the system to the initial conditions and taking a final flux measurement; compare initial flux performance to final flux performance at initial conditions.
- Ensure that choice of TMP and feed flow have corresponding retention values that are acceptable (> 0.998) at both initial and final product concentration and in each buffer[5].
- There is often very little performance difference versus feed flow rate at low product concentration. However, at the higher concentrations that will be investigated in Parts 2 and 3, the benefits of different feed flow rates should become more pronounced.

## Part 3. TMP Excursion at Final Concentration

1. Use the product from Part 2 at the final concentration in the final buffer.
2. Repeat steps 2 – 10 of Part 1.

### Calculations

Reference Part 1.

**Note:**

Reference Part 1 and Part 2 notes.

## Part 4. Diafiltration

1. Use the product from Part 3 at the optimum concentration for diafiltration; dilute as needed using the final buffer.
2. Configure the system for constant volume diafiltration.
3. Start the feed pump and achieve the optimum TMP and feed flow as determined in Part 1 and Part 3.
4. Diafilter the product with the chosen number of diavolumes:
   - Choose the number of diavolumes based on the product purity specifications (if known, see calculation below) and add a safety factor of 2 diavolumes, or
   - Use 3 – 5 diavolumes as an initial estimate for upstream UF/DF steps, or
   - Use 7 – 12 diavolumes as an initial estimate for final formulation UF/DF steps
5. Record temperatures, pressures, and flows at every diavolume; sample feed and permeate for both product retention, and retention and concentration of the contaminant of interest.
6. Plot the data as shown in Figure 6.

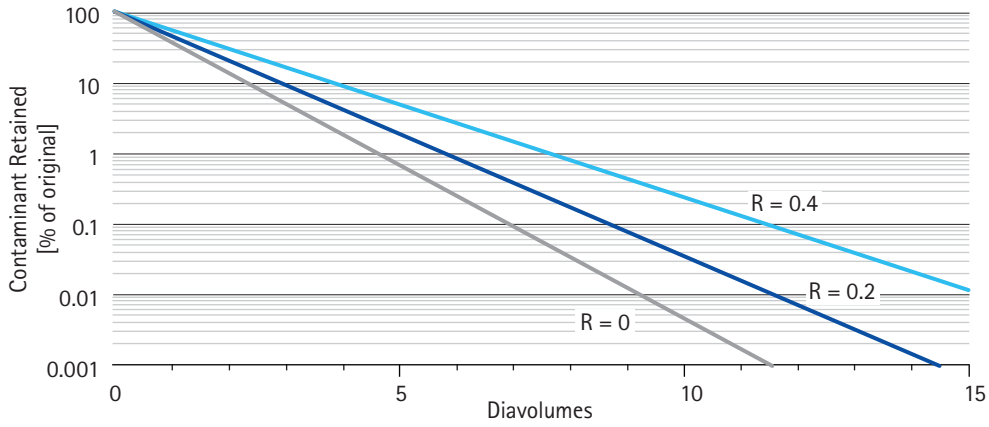



Figure 6. contaminant removal vs. Diavolumes

### Calculations

The percentage of the original contaminant in the retentate at each diavolume can be calculated from the retention values using the following:

$$\text{Remaining Contaminant [\%]} = 100 \times e^{(\text{Retention} - 1) \times N}$$

where N is the number of diavolumes.

However, since contaminant concentration is being directly measured in each feed sample throughout diafiltration, plot these concentrations as a percentage of the original and use the above equation to plot several lines of theoretical retention, as shown in Figure 6. This plot will help demonstrate the contaminant removal at various retentions.

Select the whole number of diavolumes based on the acceptable contaminant levels for the product; always add 2 – 3 diavolumes as a 10-fold safety factor for critical diafiltration steps, such as final formulation. For upstream steps, add 1 – 2 diavolumes. If the goal of diafiltration is not to wash out a contaminant but rather to reach a target pH or conductivity, then the measurement of that quality can be plotted against the number of diavolumes instead.

**Note:**

- If it appears necessary to diafilter past ~ 14 diavolumes, any dead-legs or poor mixing areas in the system will increase the apparent retention of the contaminant and make further removal difficult.
- Ensure that choice of TMP and feed flow have corresponding product retention values that are acceptable (> 0.998) throughout diafiltration.

## Paper Design and Process Simulation

The optimization parameters obtained from the previous experiments can be combined to design a full process simulation: concentration, diafiltration, (concentration,) and recovery. If time permits, a process simulation should be run immediately following the optimization work, and should employ the following:

- New set of cassettes; same membrane type, same cassette path length
- Fresh feedstock
- Fresh buffer(s)
- Optimized process parameters
- See detailed process simulation calculations below.

After performing the process simulation, the system should be cleaned with the appropriate solution according to EMD Millipore recommendations[11]. If possible, the process should be rerun using the cleaned membranes to determine the effectiveness of the cleaning cycle and the consistency of membrane performance from run-to-run. If the cleaning cycle does not prove effective, the cleaning parameters or cleaning solutions will need to be changed and the cleaning cycle will have to be tested again.

### Calculations

The membrane area can be optimized to allow the entire process (both concentration and diafiltration) to be completed in the specified timeframe (3 – 4 hours is recommended). The average flux for each concentration and diafiltration step can be estimated from the optimization data and combined with the desired volumes to be processed. The approximate required membrane area can then be calculated for both manufacturing scale and scale-down runs.

Assume an example process scenario (this would have been determined by optimization data, DF parameter, etc.):

- 2.9X Concentration:
  10 g/L to 29 g/L; flux decreases from 150 LMH to 80 LMH
- 7X Diafiltration:
  29 g/L; flux increases from 80 LMH to 85 LMH
- 3.4X Concentration:
  29 g/L to 100 g/L; flux decreases from 85 LMH to 20 LMH
- Desired process time is 4 hours

Manufacturing scale volumes as determined by the customer:

- Feed volume = 5000 L
- Retentate volume at end of 2.9X concentration = 5000 L/2.9 = 1724 L
- Permeate volume removed during 2.9X concentration = 5000 L – 1724 L = 3276 L
- 7X Diafiltration buffer volume = 7 x 1724 L = 12,068 L
- Retentate volume at end of 3.4X Concentration = 1724 L/3.4 = 507 L
- Permeate volume removed during 3.4X concentration = 1724 L – 507 L = 1217 L

**Average process flux for concentration step[13]:**

$J_{avg} = J_{final} + 0.33 (J_{initial} - J_{final}) = J_{initial} \times 0.33 + J_{final} \times 0.67$

For 2.9X concentration:

$J_{avg} = 150 \text{ LMH} \times 0.33 + 80 \text{ LMH} \times 0.67 = 103 \text{ LMH}$

For 3.4X concentration:

$J_{avg} = 85 \text{ LMH} \times 0.33 + 20 \text{ LMH} \times 0.67 = 41 \text{ LMH}$

**Average process flux for diafiltration step:**

For diafiltration the average flux can be estimated as the initial and final process flux during the diafiltration step.

**Required area:**

$\text{Area} = [(\text{Permeate volume/Average flux})_{\text{Concentration}} + (\text{Permeate volume/Average flux})_{\text{Diafiltration}} + \ldots] \text{ / Time}$

In this example:

$\text{Area} = [(3{,}276 \text{ L}/103 \text{ LMH}) + (12{,}068 \text{ L}/83 \text{ LMH}) + (1{,}217 \text{ L}/41 \text{ LMH})] / 4 \text{ hours} = 51.6 \text{ m}^2$

Add 20% safety factor:

$\text{Area} = 62 \text{ m}^2$

To perform a scale-down process simulation, the same volume to area ratio is used and scaled based on either the feed volume that can be used for the simulation or the area of the desired filtration device. For example, if the process is to be performed on one Pellicon® 2 Mini cassette (with an area of 0.1 $m^2$), then the required feed volume will be:

$\text{Scale-down feed volume} = 0.1 \text{ m}^2 \times (5000 \text{ L}/62 \text{ m}^2) = 8 \text{ L}$

Instead, if there is a specific volume of feedstock to process (example: 25 L), then the required membrane area will be:

$\text{Scale-down membrane area} = 25 \text{ L} \times (62 \text{ m}^2/5000 \text{ L}) = 0.3 \text{ m}^2$

The process parameters, including Pellicon® device type, should be consistent between scales, allowing the process to be completed in a similar timeframe with similar fluxes, pressures and loadings. The concentration factors, number of diavolumes and feed quality should be kept consistent at all scales to ensure robust scalability. However, to demonstrate process robustness and repeatability, the process should be tested at pilot scale before proceeding to manufacturing.

MASTERFLEX® B/T®

# B/T® Process Pump Systems

## Efficient fluid transfer in high-volume applications

Masterflex B/T process pump systems offer rugged design and improved performance. They handle the toughest applications, from pumping slurries, abrasives, food additives, and media, to corrosive fluids and polymers. Designed to be used with the unique PerfectPosition® tubing, these complete systems ensure reliable, continuous-duty pumping with unparalleled ease of use.


1-800-637-3739

Masterflex.com

## Rugged Design

– Cast-aluminum housing with epoxy-powder coating eliminates corrosion

– Robust gear reducer provides continuous-duty operation

– Simple latching mechanism allows easy opening and closing without the use of tools

## Easy Tube Loading

– Enhanced tubing retention secures tubing firmly in place during loading process

– Revolutionary PerfectPosition® tubing features graduated markings for foolproof tubing alignment

– No need for extra fittings (potential sources of contamination)—use a single tube length from source to delivery point

## Improved Performance

– Rotor and occlusion bed design prevents “tubing roll” during operation, extending tubing life

– Housing design eliminates any chance of over- or under-squeezing the tubing in the pump head

– PerfectPosition® tubing retention marks make it easy to determine exactly where to load the tubing to achieve optimal performance with minimal tubing wear

## Enhanced Safety

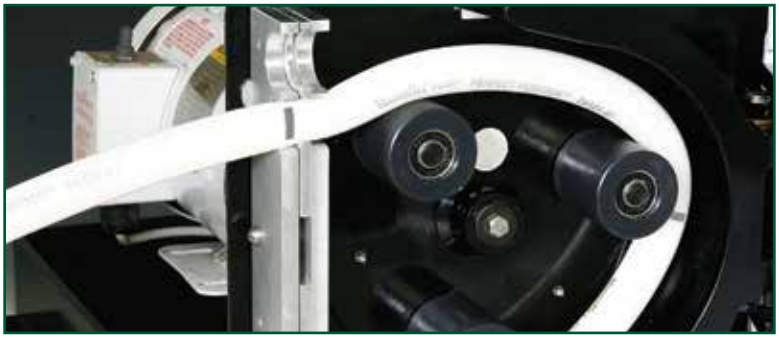
– Integrated pump interlock shuts unit down when the head is opened

– Rugged housing with window protects user from moving internal components—yet shows operator pump is working

# NO MORE GUESSING where to place your pump tubing in the pump head!

– PerfectPosition retention marks make it easy to load tubing to achieve optimal performance

– Tubing sizes ensure fully rated performance under higher pressure applications

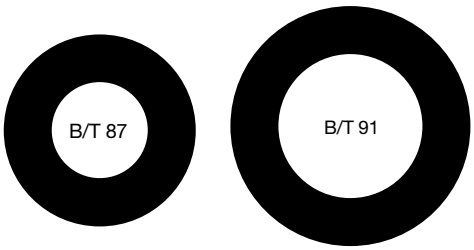


## Performance Data

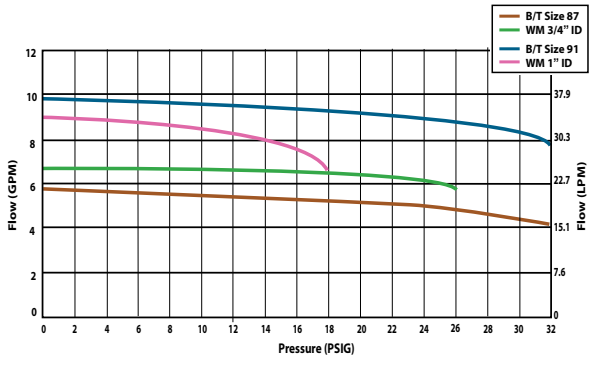
| Pump tubing size | PerfectPosition pump tubing | |
|---|---|---|
| | B/T 87 | B/T 91 |
| Inside diameter (nominal) | 0.5" (12.7 mm) | 0.75" (19.0 mm) |
| Hose barb size | ½" (12.7 mm) | ¾" (19.0 mm) |
| Flow range (approximate)* with 12 to 321 rpm drive | 0.67 to 18.9 LPM (0.17 to 5.0 GPM) | 1.4 to 42 LPM (0.4 to 11.1 GPM) |
| Maximum pressure† | 35 psi (2.4 bar) | 30 psi (2.1 bar) |
| Maximum vacuum† | 26" Hg (660 mm Hg) | |
| Suction lift† | 29 ft $H_2O$ (8.8 m $H_2O$) | |

*Determined under the following conditions: 0 psi at inlet, 0.5 psi at outlet; water temperature at 72°F (22°C).

†Actual performance varies depending on tubing formulation—values shown are for firm tubing.




## Masterflex® B/T® vs Watson-Marlow 700 Series*



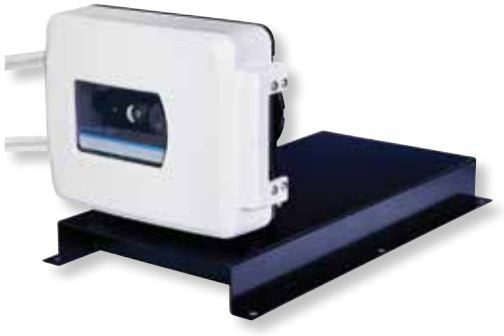

*Watson-Marlow is a trademark of Watson-Marlow Limited.

## Pump Tubing

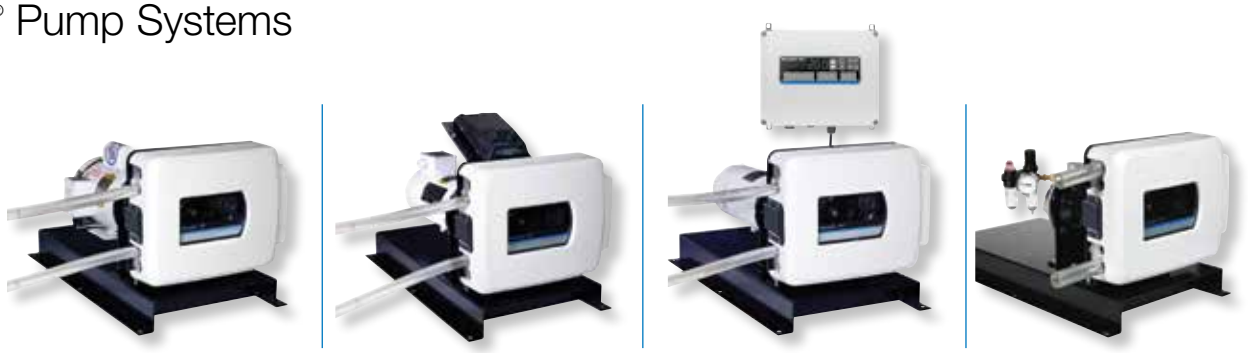
| Tubing formulation | Description | Tubing size B/T 87 | Tubing size B/T 91 |
|---|---|---|---|
| **Silicone** (platinum-cured) | • Smooth surface; low protein binding levels<br>• Fungus-resistant; phthalate- and latex-free<br>• Accommodates a wide temperature range | MK-96510-87 | MK-96510-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **Silicone** (peroxide-cured) | • Smooth surface for minimal particle entrapment<br>• Very low extractables, with documented biocompatibility for sensitive applications | MK-96400-87 | MK-96400-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **BioPharm Plus Silicone** (platinum-cured) | • Very low extractable; biocompatible for sensitive applications<br>• Longest tubing life of any silicone pump tubing<br>• Lower spallation than regular silicone | MK-96445-87 | MK-96445-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **Puri-Flex**™ | • Heat sealable, weldable, and economical<br>• Long pump life when compared to silicone or many other TPE tubings | MK-96419-87 | MK-96419-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **C-Flex®** | • Heat sealable, weldable, economical<br>• Longest life of any C-Flex formulation<br>• Better resistance to pH fluctuations | MK-06424-87 | MK-06424-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **PharMed® BPT** | • Over 10,000 hours of tubing life<br>• Resists ozone and UV radiation<br>• Ideal for tissue and cell culture work | MK-06507-87 | MK-06507-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **PharmaPure®** | • Nontoxic and nonhemolytic<br>• Great for tissue and cell work<br>• Low extractables and gas permeability | MK-06437-87 | MK-06437-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **Chem-Durance® Bio** | • Excellent chemical resistance<br>• Plasticizer-free inner liner<br>• Excellent biocompatibility | MK-06443-87 | MK-06443-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **Tygon® E-LFL** | • Longest life of all Tygon formulations<br>• USP Class VI, EU Pharmacopoeia 3.2.9, FDA 21 | MK-06440-87 | MK-06440-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **Tygon® E-Food** (B-44-4X) | • Complies with FDA, NSF, 3A, and EU food regulations<br>• Smooth inner surface unaffected by common commercial sanitizers | MK-06418-87 | MK-06418-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |
| **Norprene® Food** (A-60-F) | • Ideal for high-temperature food applications<br>• Resists heat, ozone, acids, and alkalies<br>• Complies with FDA and NSF food regulations | MK-06399-87 | MK-06399-91 |
| | | 3 m (10 ft)/pk | 3 m (10 ft)/pk |

## B/T® Pump Heads

| Motor mount | NEMA Type 56C | IEC 72/ISO 71 with B5 flange |
|---|---|---|
| Mounting type | Direct-coupled | |
| Motor size | Depends on specifications of type of motor selected (requires minimum 0.5 hp; 1800 rpm maximum) | |
| rpm | | |
| IP rating | | |
| Power | | |
| Catalog number | MK-77111-50 | MK-77111-55 |



## B/T® Pump Systems



ISO 9001:2008 CERTIFIED SUPPLIER

77111-37, -67, -77, -80

Zone 2 77111-80

77111-30, -37

Except 77111-30, -37

| | Analog | | | | Digital | | Specialty |
|---|---|---|---|---|---|---|---|
| Description | Fixed-speed | | Variable-speed | | Modular dispensing | | Air-powered |
| Flow range | 18.9 or 42 LPM | 16 or 35 LPM | 0.71 to 42 LPM | | 0.71 to 42 LPM | | 2.1 to 42 LPM |
| Motor size | 1 hp (0.75 kW) | | ½ hp (0.37 kW) | | ¾ hp (0.56 kW) | | ¾ hp (0.56 kW) |
| rpm | 321 | 271 | 12 to 321 | | 12 to 321 | | 35 to 321 |
| IP rating | IP55 | | IP56 | | IP56 | | IP56 |
| Power (VAC, Hz) | 115 (60) | 230 (50) | 115 (50/60) | 230 (50/60) | 90 to 130 (50/60) | 190 to 260 (50/60) | Requires 24 cfm (0.68 $m^3$) 100 psi (6.9 bar) minimum |
| Amps | 12.6 | 6.3 | 5 | 2.5 | 10.5 | 5.7 | — |
| Catalog number | MK-77111-30 | MK-77111-37 | MK-77111-60 | MK-77111-67 | MK-77111-70 | MK-77111-77 | MK-77111-80 |

## D. Material Safety Data Sheets

**Acetic Acid,ACS**

## SECTION 1 : Identification of the substance/mixture and of the supplier

**Product name** : **Acetic Acid,ACS**

**Manufacturer/Supplier Trade name**:

**Manufacturer/Supplier Article number**: **S25118**

**Recommended uses of the product and uses restrictions on use**:

**Manufacturer Details**:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

**Supplier Details**:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

**Emergency telephone number**:

Fisher Science Education Emergency Telephone No.: 800-535-5053

## SECTION 2 : Hazards identification

**Classification of the substance or mixture**:

**Flammable**
Flammable liquids, category 3

**Corrosive**
Serious eye damage, category 1
Skin corrosion, category 1A

Flammable liq. 3
Skin Corr. 1A
Eye Damage. 1
Acute toxicity, dermal. 4
Acute toxicity, oral. 5
Acute toxicity, inhalation. 3

**Signal word** :Danger

**Hazard statements**:
Flammable liquid and vapour
Causes severe skin burns and eye damage
May be harmful if swallowed
Toxic if inhaled
Harmful in contact with skin

**Precautionary statements**:
If medical advice is needed, have product container or label at hand
Keep out of reach of children
Read label before use
Keep away from heat/sparks/open flames/hot surfaces. No smoking
Wash skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection

Do not breathe dust/fume/gas/mist/vapours/spray
Use only outdoors or in a well-ventilated area
Keep container tightly closed
Ground/bond container and receiving equipment
Use only non-sparking tools
Take precautionary measures against static discharge
Use explosion-proof electrical/ventilating/light/equipment
IF SWALLOWED: Rinse mouth. Do NOT induce vomiting
Call a POISON CENTER or doctor/physician if you feel unwell
Take off contaminated clothing and wash before reuse
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing
Immediately call a POISON CENTER or doctor/physician
Specific treatment (see supplemental first aid instructions on this label)
In case of fire: Use agents recommended in section 5 for extinction
Store in a well ventilated place. Keep cool
Store locked up
Dispose of contents and container as instructed in Section 13

**Other Non-GHS Classification**:

**WHMIS**

B3 

D2B 

E 

**NFPA/HMIS**


NFPA SCALE (0-4)


HMIS RATINGS (0-4)

## SECTION 3 : Composition/information on ingredients

**Ingredients:**

**Acetic Acid,ACS**

| | | |
|---|---|---|
| CAS 64-19-7 | Acetic Acid, ACS | >90 % |
| | | Percentages are by weight |

## SECTION 4 : First aid measures

### Description of first aid measures

**After inhalation:** Move exposed individual to fresh air. Loosen clothing as necessary and position individual in a comfortable position.Seek medical advice if discomfort or irritation persists.Give artificial respiration if necessary. If breathing is difficult, give oxygen.

**After skin contact:** Wash affected area with soap and water. Rinse/flush exposed skin gently using water for 15-20 minutes. Seek medical attention if irritation persists or if concerned.

**After eye contact:** Protect unexposed eye. Rinse/flush exposed eye(s) gently using water for 15-20 minutes. Remove contact lens(es) if able to do so during rinsing. Seek medical attention if irritation persists or if concerned.

**After swallowing:** Rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water. Seek medical attention if irritation, discomfort or vomiting persists.

### Most important symptoms and effects, both acute and delayed:

Irritation, Nausea,Headache, Shortness of breath.;

### Indication of any immediate medical attention and special treatment needed:

If seeking medical attention, provide SDS document to physician.

## SECTION 5 : Firefighting measures

### Extinguishing media

**Suitable extinguishing agents:** Use water, dry chemical, chemical foam, carbon dioxide, or alcohol-resistant foam.

**For safety reasons unsuitable extinguishing agents:**

### Special hazards arising from the substance or mixture:

Use water spray to cool unopened containers.

### Advice for firefighters:

**Protective equipment:** Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls.When necessary use NIOSH approved breathing equipment. Wear protective eyeware, gloves, and clothing. Refer to Section 8.

**Additional information (precautions):** Do not inhale gases, fumes, dust, mist, vapor, and aerosols. Remove all sources of ignition.

## SECTION 6 : Accidental release measures

### Personal precautions, protective equipment and emergency procedures:

Ensure adequate ventilation.Use personal protective equipment. Avoid contact with skin, eyes and clothing.Remove from all sources of ignition.

### Environmental precautions:

Prevent from reaching drains, sewer or waterway. Do not let product enter drains.

### Methods and material for containment and cleaning up:

If necessary, use trained response staff/contractor.Absorb with suitable absorbent material such as sand or earth and containerize for disposal. Dispose of empty containers as unused product. Refer to Section 13.Soak with inert material.Use spark-proof tools and explosion-proof equipment.

**Reference to other sections:**

## SECTION 7 : Handling and storage

**Precautions for safe handling:**

Take measures to prevent the build up of electrostatic charge.Follow advice and precautions. Refer to Section 5.Use under a chemical fume hood.Use explosion-proof equipment.Wash hands after handling.Avoid contact with skin and eyes.Do not eat, drink, smoke, or use personal products when handling chemical substances. Use only in well ventilated areas.Do not inhale gases, fumes, dust, mist, vapor, and aerosols. Follow good hygiene procedures when handling chemical materials. Refer to Section 8.Keep away from open flames, hot surfaces, and sources of ignition.

**Conditions for safe storage, including any incompatibilities:**

Store in a cool location. Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Keep container tightly sealed.Store with like hazards. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

## SECTION 8 : Exposure controls/personal protection




| | |
|---|---|
| **Control Parameters:** | 64-19-7, Acetic acid , ACGIH TLV: 25mg/m3<br>64-19-7, Acetic acid , OSHA PEL: 25mg/m3 |
| **Appropriate Engineering controls:** | Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use or handling. Ensure that dust-handling systems (exhaust ducts, dust collectors, vessels, and processing equipment) are designed to prevent the escape of dust into the work area.Use chemical fume hood.Use explosion-proof equipment. |
| **Respiratory protection:** | Not required under normal conditions of use. Use suitable respiratory protective device when high concentrations are present. |
| **Protection of skin:** | Select glove material impermeable and resistant to the substance.Select glove material based on rates of diffusion and degradation. |
| **Eye protection:** | Safety goggles with face shield. |
| **General hygienic measures:** | Wash hands before breaks and at the end of work. Avoid contact with the eyes and skin.Perform routine housekeeping.Follow proper handling methods. Refer to Section 6.Follow proper handling methods. Refer to Section 7. |

## SECTION 9 : Physical and chemical properties

| | | | |
|---|---|---|---|
| **Appearance (physical state,color)**: | Clear colorless liquid | **Explosion limit lower**:<br>**Explosion limit upper**: | 4 %<br>19.9 % |
| **Odor**: | Pungent Vinegar | **Vapor pressure**: | 73.3 hPa (55.0 mmHg) at 50.0°C/ 15.2 hPa (11.4 mmHg) at 20.0°C |
| **Odor threshold**: | Not Available | **Vapor density**: | Not Available |
| **pH-value**: | 2.4 @ 60.05 g/l | **Relative density**: | 1.049 g/cm3 at 25 °C |

| | | | |
|---|---|---|---|
| **Melting/Freezing point**: | 16.2°C | **Solubilities**: | Completely soluble |
| **Boiling point/Boiling range**: | 117 - 118°C | **Partition coefficient (n-octanol/water)**: | log Pow: -0.17 |
| **Flash point (closed cup)**: | Not Available | **Auto/Self-ignition temperature**: | 485.0°C |
| **Evaporation rate**: | Not Available | **Decomposition temperature**: | Not Available |
| **Flammability (solid,gaseous)**: | Not Available | **Viscosity**: | a. Kinematic:Not Available<br>b. Dynamic: Not Available |
| **Density**: Not Available | | | |

## SECTION 10 : Stability and reactivity

**Reactivity:**Nonreactive under normal conditions.
**Chemical stability:**Stable under normal conditions.
**Possible hazardous reactions:**None under normal processing
**Conditions to avoid:**Moisture sensitive.Heat, flames and sparks.Incompatible Materials.
**Incompatible materials:**Oxidizing agents, Soluble carbonates and phosphates, Hydroxides, Metals, Peroxides, Permanganates, Potassium permanganate, Amines, Alcohols, and Nitric acid.Strong bases, strong oxidizers, metals.
**Hazardous decomposition products:**Oxides of carbon.

## SECTION 11 : Toxicological information

| | | |
|---|---|---|
| **Acute Toxicity**: | | |
| **Oral**: | | LD50 Rat: 3,310 mg/kg |
| **Dermal**: | | LD50 Rabbit: 1,112 mg/kg |
| **Inhalation**: | | LC50 Rat: 11.4 mg/l - 4 h |
| **Chronic Toxicity**: No additional information. | | |
| **Corrosion Irritation**: | | |
| **Ocular**: | | Eyes - rabbit Result: Corrosive to eyes |
| **Sensitization**: | | No additional information. |
| **Single Target Organ (STOT)**: | | No additional information. |
| **Numerical Measures**: | | No additional information. |
| **Carcinogenicity**: | | No additional information. |
| **Mutagenicity**: | | No additional information. |
| **Reproductive Toxicity**: | | Experiments have shown reproductive toxicity effects on laboratory animals. |

## SECTION 12 : Ecological information

**Ecotoxicity**

**Aquatic Tox.**: Toxicity to fish semi-static test LC50 - Oncorhynchus mykiss (rainbow trout) - > 1,000 mg/l - 96 h (OECD Test Guideline 203)

**Aquatic Tox.**: Toxicity to daphnia and other aquatic invertebrates EC50 - Daphnia magna (Water flea) - > 300.82 mg/l - 48 h (OECD Test Guideline 202)

**Persistence and degradability**: Readily biodegradable.
**Bioaccumulative potential**:
**Mobility in soil**: Aqueous solution has high mobility in soil.
**Other adverse effects**:

## SECTION 13 : Disposal considerations

**Waste disposal recommendations**:

It is the responsibility of the waste generator to properly characterize all waste materials according to applicable regulatory entities (US 40CFR262.11). Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company.Contact a licensed professional waste disposal service to dispose of this material.

## SECTION 14 : Transport information

**UN-Number**

2789

**UN proper shipping name**

Acetic acid, glacial

**Transport hazard class(es)**


**Class:**
3 Flammable liquids

**Packing group**:II
**Environmental hazard**:
**Transport in bulk**:
**Special precautions for user**:

## SECTION 15 : Regulatory information

**United States (USA)**

**SARA Section 311/312 (Specific toxic chemical listings)**:

Acute, Chronic, Fire

**SARA Section 313 (Specific toxic chemical listings)**:

None of the ingredients is listed

**RCRA (hazardous waste code)**:

None of the ingredients is listed

**TSCA (Toxic Substances Control Act)**:

All ingredients are listed.

**CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act)**:

64-19-7 Acetic Acid 5000 lb

**Proposition 65 (California)**:

**Chemicals known to cause cancer**:

None of the ingredients is listed

**Chemicals known to cause reproductive toxicity for females**:

None of the ingredients is listed

**Chemicals known to cause reproductive toxicity for males**:

None of the ingredients is listed

**Chemicals known to cause developmental toxicity**:

None of the ingredients is listed

**Canada**

**Canadian Domestic Substances List (DSL)**:

All ingredients are listed.

**Canadian NPRI Ingredient Disclosure list (limit 0.1%)**:

None of the ingredients is listed

**Canadian NPRI Ingredient Disclosure list (limit 1%)**:

64-19-7 Acetic Acid

## SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the SDS contains all the information required by the Controlled Products Regulations.Note:. The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.

**GHS Full Text Phrases**:

**Abbreviations and acronyms**:

IMDG: International Maritime Code for Dangerous Goods
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)

**Effective date** : 01.06.2015
**Last updated** : 03.27.2015

# Safety Data Sheet

## SECTION 1: Identification

### 1.1. Product Identifier

**Trade Name or Designation:** Imidazole, Reagent (Glyoxaline)
**Product Number:** 2-21660
**Other Identifying Product Numbers:** 2-21660-1, 2-21660-100G

### 1.2. Recommended Use and Restrictions on Use

General Laboratory Reagent

### 1.3. Details of the Supplier of the Safety Data Sheet

**Company:** Reagents Inc.

**Address:** 4746 Sweden Road
Charlotte, NC 28224 USA
**Telephone:** 800-732-8484

### 1.4. Emergency Telephone Number (24 hr)

CHEMTREC (USA) 800-424-9300
CHEMTREC (International) 1+ 703-527-3887

## SECTION 2: Hazard(s) Identification

### 2.1. Classification of the Substance or Mixture (in accordance with OSHA HCS 29 CFR 1910.1200)

*For the full text of the Hazard and Precautionary Statements listed below, see Section 16.*

| Hazard Class | Category | Hazard Statement | Precautionary Statements |
|---|---|---|---|
| Acute Toxicity - Oral | Category 3 | H301 | P264, P270, P301+P310, P321, P330, P405, P501 |
| Skin Corrosion / Irritation | Category 1 | H314 | P260, P264, P280, P301+P330+P331, P303+P361+P353, P363, P304+P340, P310, P321, P305+P351+P338, P405, P501 |
| Eye Damage / Irritation | Category 1 | H318 | P280, P305+P351+P338, P310 |
| Reproductive Toxicity | Category 2 | H361 | P201, P202, P280, P308+P313, P405, P501 |
| Specific Target Organs/Systemic Toxicity Following Single Exposure | Category 2 | H371 | P260, P264, P270, P308+P311, P405, P501 |
| Corrosive to Metals | Category 1 | H290 | P234, P390, P406 |

# Safety Data Sheet

## 2.2. GHS Label Elements

**Pictograms:**

**Signal Word:** **Danger**

**Hazard Statements:**

| Hazard Number | Hazard Statement |
|---|---|
| H290 | May be corrosive to metals. |
| H301 | Toxic if swallowed. |
| H314 | Causes severe skin burns and eye damage. |
| H318 | Causes serious eye damage. |
| H361 | Suspected of damaging fertility or the unborn child. |
| H371 | May cause damage to organs. |

# Safety Data Sheet

**Precautionary Statements:**

| Precautionary Number | Precautionary Statement |
|---|---|
| P201 | Obtain special instructions before use. |
| P202 | Do not handle until all safety precautions have been read and understood. |
| P234 | Keep only in original container. |
| P260 | Do not breathe dust, fumes or mist. |
| P264 | Wash arms, hands and face thoroughly after handling. |
| P270 | Do not eat, drink or smoke when using this product. |
| P280 | Wear protective gloves and eye protection. |
| P301+P310 | IF SWALLOWED: Immediately call a POISON CENTER or physician. |
| P301+P330+P331 | IF SWALLOWED: rinse mouth. Do NOT induce vomiting. |
| P303+P361+P353 | IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. |
| P305+P351+P338 | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| P308+P311 | IF exposed or concerned: Call a POISON CENTER or physician. |
| P308+P313 | IF exposed or concerned: Get medical attention. |
| P310 | Immediately call a POISON CENTER or physician. |
| P321 | Specific treatment (Wash areas of contact with water). |
| P330 | Rinse mouth. |
| P363 | Wash contaminated clothing before reuse. |
| P390 | Absorb spillage to prevent material damage. |
| P405 | Store locked up. |
| P406 | Store in corrosive resistant container with a resistant inner liner. |
| P501 | Dispose of contents in accordance with local, state, federal and international regulations. |

### 2.3. WHMIS Classification

WHMIS classification is not included based on the recommended option (Option 4) found in the Canada Gazette Part II, Vol. 149, No.3, page 458

### 2.4. Hazards not Otherwise Classified or Covered by GHS

Data not available.

## SECTION 3: Composition / Information on Ingredients

### 3.1. Components of Substance or Mixture

| Chemical Name | Formula | Molecular Weight | CAS Number | Weight% |
|---|---|---|---|---|
| Imidazole | NHCH:NCH:CH | 68.07 g/mol | 288-32-4 | 100.00% |

# Safety Data Sheet

## SECTION 4: First-Aid Measures

### 4.1. General First Aid Information

**Eye Contact:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

**Inhalation:** Not expected to require first aid. If necessary, remove to fresh air.

**Skin Contact:** IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.

**Ingestion:** IF SWALLOWED: rinse mouth. Do NOT induce vomiting.

### 4.2. Most Important Symptoms and Effects, Acute and Delayed

May cause mild irritation to areas of contact.

### 4.3. Medical Attention or Special Treatment Needed

Immediately call a POISON CENTER or physician. Specific treatment (Wash areas of contact with water).

## SECTION 5: Fire-Fighting Measures

### 5.1. Extinguishing Media

Not considered to be a fire or explosion hazard.

### 5.2. Specific Hazards Arising from the Substance or Mixture

Not considered to be a fire or explosion hazard.

### 5.3. Special Protective Equipment for Firefighters

Wear protective clothing and NIOSH-approved breathing equipment appropriate for the surrounding fire.

## SECTION 6: Accidental Release Measures

### 6.1. Personal Precautions, Protective Equipment and Emergency Procedures

Wear protective gloves and eye protection.

### 6.2. Cleanup and Containment Methods and Materials

Absorb with suitable material and dispose of in accordance with local regulations.

## SECTION 7: Handling and Storage

### 7.1. Precautions for Safe Handling and Storage Conditions

Store in corrosive resistant container with a resistant inner liner.

# Safety Data Sheet

## SECTION 8: Exposure Controls / Personal Protection

### 8.1. Control Parameters

| Chemical Name | Limit Type | Country | Exposure Limit | Information Source |
|---|---|---|---|---|
| Data not available. () | | | | |

### 8.2. Exposure Controls

**Engineering Controls:** No specific controls are needed. Normal room ventilation is adequate.

**Respiratory Protection:** No specific controls are needed. Normal room ventilation is adequate

**Skin Protection:** Wear protective gloves and eye protection.

**Eye Protection:** Wear protective gloves and eye protection.

### 8.3. Personal Protective Equipment

Wear protective gloves and eye protection.

# Safety Data Sheet

## SECTION 9: Physical and Chemical Properties

### 9.1. Basic Physical and Chemical Properties

| | |
|---|---|
| **Appearance:** | White to off-white solid |
| **Physical State:** | Solid |
| **Odor:** | Data not available. |
| **Odor Threshold:** | Data not available. |
| **pH:** | Data not available. |
| **Melting/Freezing Point:** | 88 - 90°C |
| **Initial Boiling Point /Range:** | Data not available. |
| **Flash Point:** | 145°C closed cup [DIN 51758] |
| **Evaporation Rate:** | Data not available. |
| **Flammability:** | Data not available. |
| **Flammability/Explosive Limits:** | Data not available. |
| **Vapor Pressure:** | 0.003 hPa at 20°C |
| **Vapor Density:** | Data not available. |
| **Relative Density:** | 1.03 |
| **Solubility:** | 633 G/L at 20°C |
| **Partition Coeffieient (n-Octanol/Water):** | -0.02 |
| **Auto-Ignition Temperature:** | Data not available. |
| **Decomposition Temperature:** | Data not available. |
| **Viscosity:** | Data not available. |
| **ExplosiveProperties:** | Data not available. |
| **Oxidizing Properties:** | Data not available. |

## SECTION 10: Stability and Reactivity

### 10.1. Reactivity and Chemical Stability

Stable under normal conditions of use and storage.

### 10.2. Possibility of Hazardous Reactions

Data not available.

### 10.3. Conditions to Avoid and Incompatible Materials

Keep only in original container.

### 10.4. Hazardous Decomposition Products

May emit irritating fumes when heated to decomposition.

# Safety Data Sheet

## SECTION 11: Toxicological Information

### 11.1. Information on Toxicological Effects

**Acute Toxicity - Oral Exposure:**

Toxic if swallowed. Wash arms, hands and face thoroughly after handling. Do not eat, drink or smoke when using this product. IF SWALLOWED: Immediately call a POISON CENTER or physician. Specific treatment (Wash areas of contact with water). Rinse mouth. Store locked up. Dispose of contents in accordance with local, state, federal and international regulations.

**Acute Toxicity - Dermal Exposure:**

Not applicable.

**Acute Toxicity - Inhalation Exposure:**

Not applicable.

**Acute Toxicity - Other Information:**

Data not available.

**Skin Corrosion and Irritation:**

Causes severe skin burns and eye damage. Do not breathe dust, fumes or mist. Wash arms, hands and face thoroughly after handling. Wear protective gloves and eye protection. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. Wash contaminated clothing before reuse. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER or physician. Specific treatment (Wash areas of contact with water). IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Store locked up. Dispose of contents in accordance with local, state, federal and international regulations.

**Serious Eye Damage and Irritation:**

Causes serious eye damage. Wear protective gloves and eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

**Respiratory Sensitization:**

Not applicable.

**Skin Sensitization:**

Not applicable.

**Germ Cell Mutagenicity:**

Not applicable.

**Carcinogenicity:**

Not applicable.

**Reproductive Toxicity:**

Suspected of damaging fertility or the unborn child. Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Wear protective gloves and eye protection. IF exposed or concerned: Get medical attention. Store locked up. Dispose of contents in accordance with local, state, federal and international regulations.

**Specific Target Organ Toxicity from Single Exposure:**

May cause damage to organs. Do not breathe dust, fumes or mist. Wash arms, hands and face thoroughly after handling. Do not eat, drink or smoke when using this product. IF exposed or concerned: Call a POISON CENTER or physician. Store locked up. Dispose of contents in accordance with local, state, federal and international regulations.

**Specific Target Organ Toxicity from Repeated Exposure:**

Not applicable.

**Aspiration Hazard:**

Not applicable.

**Additional Toxicology Information:**

Data not available.

## SECTION 12: Ecological Information

### 12.1. Ecotoxicity

Not applicable.

### 12.2. Persistence and Degradability

Data not available.

### 12.3. Bioaccumulative Potential

Data not available.

### 12.4. Mobility in Soil

Data not available.

### 12.5. Other Adverse Ecological Effects

Data not available.

## SECTION 13: Disposal Considerations

### 13.1. Waste Treatment Methods

Data not available.

# Safety Data Sheet

## SECTION 14: Transportation Information

### 14.1. Transportation by Land - Department of Transportation (DOT, United States of America)

**UN Number:** UN3263

**Proper Shipping Name:** Corrosive solid, basic, organic, nos (Imidazole)

**Hazard Class:** 8

**Packing Group:** III

**Hazard Placard Labels:**


### 14.2. Transportation by Air - International Air Transport Association (IATA)

**UN Number:** UN3263

**Proper Shipping Name:** Corrosive solid, basic, organic, nos (Imidazole)

**Hazard Class:** 8

**Packing Group:** III

**Hazard Placard Labels:**


## SECTION 15: Regulatory Information

### 15.1. Occupational Safety and Health Administration (OSHA) Hazards

Not listed.

### 15.2. Superfund Amendments and Reauthorization Act (SARA) 302 Extremely Hazardous Substances

Not listed.

### 15.3. Superfund Amendments and Reauthorization Act (SARA) 311/312 Hazardous Chemicals

Not listed.

### 15.4. Superfund Amendments and Reauthorization Act (SARA) 313 Toxic Release Inventory (TRI)

Not listed.

### 15.5. Massachusetts Right-to-Know Substance List

Not listed.

### 15.6. Pennsylvania Right-to-Know Hazardous Substances

Not listed.

### 15.7. New Jersey Worker and Community Right-to-Know Components

Not listed.

### 15.8. California Proposition 65

Not listed.

### 15.9. Canada Domestic Substances List / Non-Domestic Substances List (DSL/NDSL)

Imidazole (CAS # 288-32-4): Present

### 15.10. United States of America Toxic Substances Control Act (TSCA) List

Imidazole (CAS # 288-32-4): Present

### 15.11. European Inventory of Existing Commercial Chemical Substances (EINECS), European List of Notified Chemical Substances (ELINCS), and No Longer Polymers (NLP)

Not listed.

## SECTION 16: Other Information

### 16.1. Full Text of Hazard Statements and Precautionary Statements

May be corrosive to metals. Toxic if swallowed. Causes severe skin burns and eye damage. Causes serious eye damage. Suspected of damaging fertility or the unborn child. May cause damage to organs.

Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Keep only in original container. Do not breathe dust, fumes or mist. Wash arms, hands and face thoroughly after handling. Do not eat, drink or smoke when using this product. Wear protective gloves and eye protection.

IF SWALLOWED: Immediately call a POISON CENTER or physician. IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. IF INHALED: Remove person to fresh air and keep comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Call a POISON CENTER or physician. Specific treatment (Wash areas of contact with water). Wash contaminated clothing before reuse. Absorb spillage to prevent material damage.

Store locked up. Store in corrosive resistant container with a resistant inner liner.

Dispose of contents in accordance with local, state, federal and international regulations.

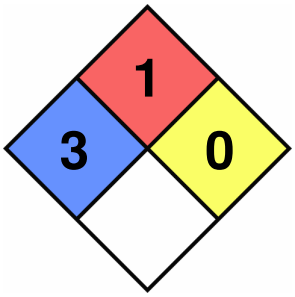
# Safety Data Sheet

## 16.2. Miscellaneous Hazard Classes

**Canadian Carcinogenicity Hazard Class:** Not Applicable.
**Physical Hazards Not Otherwise Classified (PHNOC):** Not Applicable.
**Health Hazards Not Otherwise Classified (HHNOC):** Not Applicable.
**Biohazardous Infectious Materials Hazard Class:** Not Applicable.

## 16.3. National Fire Protection Association (NFPA) Rating

**Health:** 3
**Flammability:** 1
**Reactivity:** 0
**Special Hazard:**




## 16.4. Document Revision

**Last Revision Date:** 8/31/2015

## DISCLAIMER

When handled properly by qualified personnel, the product described herein does not present a significant health or safety hazard. Alteration of its characteristics by concentration, evaporation, addition of other substances, or other means may present hazards not specifically addressed herein and which must be evaluated by the user. The information furnished herein is believed to be accurate and represents the best data currently available to us. No warranty, expressed or implied, is made and REAGENTS, INC. assumes no legal responsibility or liability whatsoever resulting from its use.

**Sodium Acetate,Anhydrous,**

## SECTION 1 : Identification of the substance/mixture and of the supplier

**Product name** : **Sodium Acetate,Anhydrous,**

**Manufacturer/Supplier Trade name**:

**Manufacturer/Supplier Article number**: **S25530**

**Recommended uses of the product and uses restrictions on use**:

**Manufacturer Details**:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

**Supplier Details**:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

**Emergency telephone number**:

Fisher Science Education Emergency Telephone No.: 800-535-5053

## SECTION 2 : Hazards identification

**Classification of the substance or mixture**:

Not classified for physical or health hazards under GHS.
Hazards Not Otherwise Classified - Combustible Dust

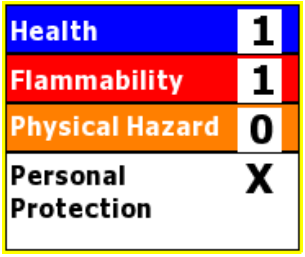
**Hazard statements**:
**Precautionary statements**:
If medical advice is needed, have product container or label at hand
Keep out of reach of children
Read label before use
Do not eat, drink or smoke when using this product

**Combustible Dust Hazard: :**
May form combustible dust concentrations in air (during processing).

**Other Non-GHS Classification**:

**WHMIS**
**NFPA/HMIS**


NFPA SCALE (0-4)




HMIS RATINGS (0-4)

## SECTION 3 : Composition/information on ingredients

| **Ingredients:** | | |
|---|---|---|
| CAS 127-09-3 | Sodium Acetate, Anhydrous, ACS | 100 % |
| | | Percentages are by weight |

## SECTION 4 : First aid measures

### Description of first aid measures

**After inhalation:** Move exposed individual to fresh air. Loosen clothing as necessary and position individual in a comfortable position.Seek medical advice if discomfort or irritation persists.If breathing difficult, give oxygen.

**After skin contact:** Wash affected area with soap and water. Rinse/flush exposed skin gently using water for 15-20 minutes. Seek medical advice if discomfort or irritation persists.

**After eye contact:** Protect unexposed eye. Rinse/flush exposed eye(s) gently using water for 15-20 minutes. Remove contact lens(es) if able to do so during rinsing. Seek medical attention if irritation persists or if concerned.

**After swallowing:** Rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water. Seek medical attention if irritation, discomfort or vomiting persists.

### Most important symptoms and effects, both acute and delayed:

Irritation, Nausea,Headache, Shortness of breath.;

### Indication of any immediate medical attention and special treatment needed:

If seeking medical attention, provide SDS document to physician.

## SECTION 5 : Firefighting measures

### Extinguishing media

**Suitable extinguishing agents:** Water spray. Dry chemical powder. Carbon dioxide. Alcohol foam. Polymer foam. If in laboratory setting, follow laboratory fire suppression procedures. Use appropriate fire suppression agents for adjacent combustible materials or sources of ignition

**For safety reasons unsuitable extinguishing agents:**

### Special hazards arising from the substance or mixture:

Combustion products may include carbon oxides or other toxic vapors.Thermal decomposition can lead to release of irritating gases and vapors.Avoid generating dust; fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard.

### Advice for firefighters:

**Protective equipment:** Use NIOSH-approved respiratory protection/breathing apparatus.

**Additional information (precautions):** Move product containers away from fire or keep cool with water spray as a protective measure, where feasible.Use spark-proof tools and explosion-proof equipment.

## SECTION 6 : Accidental release measures

### Personal precautions, protective equipment and emergency procedures:

Wear protective equipment. Transfer to a disposal or recovery container.Use spark-proof tools and explosion-proof equipment.Use respiratory protective device against the effects of fumes/dust/aerosol. Keep unprotected persons away. Ensure adequate ventilation.Keep away from ignition sources. Protect from heat.Stop the spill, if possible. Contain spilled material by diking or using inert absorbent.

### Environmental precautions:

Prevent from reaching drains, sewer or waterway. Collect contaminated soil for characterization per Section 13

### Methods and material for containment and cleaning up:

If in a laboratory setting, follow Chemical Hygiene Plan procedures.Place into properly labeled containers for recovery or disposal. If necessary, use trained response staff/contractor.Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Avoid dispersal of dust in the air (i.e., clearing dust surfaces with compressed air). Collect solids in powder form using vacuum with (HEPA filter)

**Reference to other sections:**

## SECTION 7 : Handling and storage

**Precautions for safe handling:**

Minimize dust generation and accumulation. Wash hands after handling. Avoid dispersal of dust in the air (i.e., clearing dust surfaces with compressed air). Routine housekeeping should be instituted to ensure that dusts do not accumulate on surfaces. Dry powders can build static electricity charges when subjected to the friction of transfer and mixing operations. Follow good hygiene procedures when handling chemical materials. Do not eat, drink, smoke, or use personal products when handling chemical substances. If in a laboratory setting, follow Chemical Hygiene Plan.Use only in well ventilated areas.Avoid generation of dust or fine particulate.Avoid contact with eyes, skin, and clothing.

**Conditions for safe storage, including any incompatibilities:**

Store in a cool location. Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store away from foodstuffs. Store away from oxidizing agents.Store in cool, dry conditions in well sealed containers. Keep container tightly sealed.Store with like hazards

## SECTION 8 : Exposure controls/personal protection

| | |
|---|---|
| **Control Parameters:** | , , OSHA PEL TWA (Total Dust) 15 mg/m3 (50 mppcf*)<br>, , ACGIH TLV TWA (inhalable particles) 10 mg/m3 |
| **Appropriate Engineering controls:** | Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use/handling.Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapor or dusts (total/respirable) below the applicable workplace exposure limits (Occupational Exposure Limits-OELs) indicated above.Use under a fume hood. It is recommended that all dust control equipment such as local exhaust ventilation and material transport systems involved in handling of this product contain explosion relief vents or an explosion suppression system or an oxygen deficient environment.Ensure that dust-handling systems (such as exhaust ducts, dust collectors, vessels, and processing equipment) are designed in a manner to prevent the escape of dust into the work area (i.e., there is no leakage from the equipment). |
| **Respiratory protection:** | Not required under normal conditions of use. Use suitable respiratory protective device when high concentrations are present. Use suitable respiratory protective device when aerosol or mist is formed. For spills, respiratory protection may be advisable. |
| **Protection of skin:** | The glove material has to be impermeable and resistant to the product/ the substance/ the preparation being used/handled.Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation. |
| **Eye protection:** | Safety glasses with side shields or goggles. |

**General hygienic measures:** The usual precautionary measures are to be adhered to when handling chemicals. Keep away from food, beverages and feed sources. Immediately remove all soiled and contaminated clothing. Wash hands before breaks and at the end of work. Do not inhale gases/fumes/dust/mist/vapor/aerosols. Avoid contact with the eyes and skin.

## SECTION 9 : Physical and chemical properties

| | | | |
|---|---|---|---|
| **Appearance (physical state,color)**: | White solid | **Explosion limit lower**:<br>**Explosion limit upper**: | Not Determinedn<br>Not Determined |
| **Odor**: | Odorless to slight acetic odor | **Vapor pressure**: | Not Determined |
| **Odor threshold**: | Not Determined | **Vapor density**: | Not Determined |
| **pH-value**: | Not Determined | **Relative density**: | Not Determined |
| **Melting/Freezing point**: | 324 C | **Solubilities**: | Very soluble. 1190g/L (20 C) |
| **Boiling point/Boiling range**: | Not Determined | **Partition coefficient (n-octanol/water)**: | Not Determined |
| **Flash point (closed cup)**: | Not Determined | **Auto/Self-ignition temperature**: | Not Determined |
| **Evaporation rate**: | Not Determined | **Decomposition temperature**: | Not Determined |
| **Flammability (solid,gaseous)**: | Not Determined | **Viscosity**: | a. Kinematic:Not Determined<br>b. Dynamic: Not Determined |
| **Density**: Not Determinedn<br>**Additional property::**Hygroscopic<br>**Specific Gravity: :**Approx. 1.8 | | | |

## SECTION 10 : Stability and reactivity

**Reactivity:**Nonreactive under normal conditions.
**Chemical stability:**No decomposition if used and stored according to specifications.Hydroscopic.
**Possible hazardous reactions:**Explosive mixture may form with fluorine and potassium nitrite
**Conditions to avoid:**Store away from oxidizing agents, strong acids or bases.
**Incompatible materials:**Strong oxidizing agents. Strong acids.Strong bases.
**Hazardous decomposition products:**Carbon oxides (CO, CO2).Oxides of sodium

## SECTION 11 : Toxicological information

| **Acute Toxicity**: | | |
|---|---|---|
| **Oral**: | LD50: 3530mg/kg (rat) | Sodium Acetate (127-09-3) |
| **Inhalation**: | >30 g/m3 1 h | Inhalation LC50 Rat |
| **Dermal**: | >10 g/kg | Dermal LD50 Rabbit |

| | |
|---|---|
| **Chronic Toxicity**: No additional information. | |
| **Corrosion Irritation**: No additional information. | |
| **Sensitization**: | No additional information. |
| **Single Target Organ (STOT)**: | No additional information. |
| **Numerical Measures**: | No additional information. |
| **Carcinogenicity**: | No additional information. |
| **Mutagenicity**: | No additional information. |
| **Reproductive Toxicity**: | No additional information. |

## SECTION 12 : Ecological information

**Ecotoxicity**

**Water Flea**: 48 Hr EC50 Daphnia magna: >1000 mg/L

**Persistence and degradability**: Readily degradable in the environment.
**Bioaccumulative potential**:
**Mobility in soil**:
**Other adverse effects**:

## SECTION 13 : Disposal considerations

**Waste disposal recommendations**:

Product/containers must not be disposed together with household garbage. Do not allow product to reach sewage system or open water.It is the responsibility of the waste generator to properly characterize all waste materials according to applicable regulatory entities (US 40CFR262.11). Consult federal state/ provincial and local regulations regarding the proper disposal of waste material that may incorporate some amount of this product.

## SECTION 14 : Transport information

**UN-Number**

Not Regulated

**UN proper shipping name**

Not Regulated

**Transport hazard class(es)**
**Packing group**:Not Regulated
**Environmental hazard**:
**Transport in bulk**:
**Special precautions for user**:

## SECTION 15 : Regulatory information

**United States (USA)**

**SARA Section 311/312 (Specific toxic chemical listings)**:

None of the ingredients is listed

**SARA Section 313 (Specific toxic chemical listings)**:

None of the ingredients is listed

**RCRA (hazardous waste code)**:

None of the ingredients is listed

**TSCA (Toxic Substances Control Act)**:

All ingredients are listed.

**CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act)**:

None of the ingredients is listed

**Proposition 65 (California)**:

**Chemicals known to cause cancer**:

None of the ingredients is listed

**Chemicals known to cause reproductive toxicity for females**:

None of the ingredients is listed

**Chemicals known to cause reproductive toxicity for males**:

None of the ingredients is listed

**Chemicals known to cause developmental toxicity**:

None of the ingredients is listed

**Canada**

**Canadian Domestic Substances List (DSL)**:

All ingredients are listed.

**Canadian NPRI Ingredient Disclosure list (limit 0.1%)**:

None of the ingredients is listed

**Canadian NPRI Ingredient Disclosure list (limit 1%)**:

None of the ingredients is listed

## SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the SDS contains all the information required by the Controlled Products Regulations.Note:. The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.

**GHS Full Text Phrases**:

**Abbreviations and acronyms**:

IMDG: International Maritime Code for Dangerous Goods
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)

DOT: US Department of Transportation
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)

**Effective date** : 01.06.2015
**Last updated** : 03.19.2015

# Sodium Hydroxide,1M

## SECTION 1 : Identification of the substance/mixture and of the supplier

**Product name** : **Sodium Hydroxide,1M**

**Manufacturer/Supplier Trade name**:

**Manufacturer/Supplier Article number**: **S25549A**

**Recommended uses of the product and uses restrictions on use**:

**Manufacturer Details**:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

**Supplier Details**:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

**Emergency telephone number**:

Fisher Science Education Emergency Telephone No.: 800-535-5053

## SECTION 2 : Hazards identification

**Classification of the substance or mixture**:

**Corrosive**
Corrosive to metals, category 1
Skin corrosion, category 1B
Serious eye damage, category 1

Skin Corr. 1B
Eye corr. 1
Metal Corr. 1

**Signal word** :Danger

**Hazard statements**:
May be corrosive to metals
Causes severe skin burns and eye damage
Causes serious eye damage

**Precautionary statements**:
If medical advice is needed, have product container or label at hand
Keep out of reach of children
Read label before use
Keep only in original container
Wash ... thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection
Do not breathe dust/fume/gas/mist/vapours/spray
IF SWALLOWED: Rinse mouth. Do NOT induce vomiting
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
Wash contaminated clothing before reuse
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
IF INHALED: Call a POISON CENTER or doctor/physician if you feel unwell
Immediately call a POISON CENTER or doctor/physician
Specific treatment (see supplemental first aid instructions on this label)

Absorb spillage to prevent material damage
Store in a corrosive resistant/... container with a resistant inner liner
Store locked up
Dispose of contents/container to ...

**Other Non-GHS Classification**:

**WHMIS**

E 

**NFPA/HMIS**


NFPA SCALE (0-4)

| | |
|---|---|
| Health | 2 |
| Flammability | 0 |
| Physical Hazard | 0 |
| Personal Protection | X |

HMIS RATINGS (0-4)

## SECTION 3 : Composition/information on ingredients

| **Ingredients:** | | |
|---|---|---|
| CAS 1310-73-2 | Sodium Hydroxide | 4 % |
| CAS 7732-18-5 | Deionized Water | 96 % |
| | | Percentages are by weight |

## SECTION 4 : First aid measures

**Description of first aid measures**

**After inhalation:** Move exposed individual to fresh air. Loosen clothing as necessary and position individual in a comfortable position.Seek medical advice if discomfort or irritation persists.If breathing difficult, give oxygen.

**After skin contact:** Take off contaminated clothing and shoes immediately.Wash affected area with soap and water.Seek medical attention if irritation, discomfort persist.

**After eye contact:** Protect unexposed eye. Rinse/flush exposed eye(s) gently using water for 15-20 minutes. Remove contact lens(es) if able to do so during rinsing. Immediately get medical assistance.

**After swallowing:** Rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water. Seek medical attention if irritation, discomfort or vomiting persists.

**Most important symptoms and effects, both acute and delayed:**

Irritation, Nausea,Headache, Shortness of breath.;

**Indication of any immediate medical attention and special treatment needed:**

If seeking medical attention, provide SDS document to physician.

## SECTION 5 : Firefighting measures

**Extinguishing media**

**Suitable extinguishing agents:** If in laboratory setting, follow laboratory fire suppression procedures. Use appropriate fire suppression agents for adjacent combustible materials or sources of ignition

**For safety reasons unsuitable extinguishing agents:** Carbon dioxide.Carbon dioxide.

**Special hazards arising from the substance or mixture:**

Combustion products may include carbon oxides or other toxic vapors.Thermal decomposition can lead to release of irritating gases and vapors.Sodium oxides.

**Advice for firefighters:**

**Protective equipment:** Use NIOSH-approved respiratory protection/breathing apparatus.

**Additional information (precautions):** Move product containers away from fire or keep cool with water spray as a protective measure, where feasible.

## SECTION 6 : Accidental release measures

**Personal precautions, protective equipment and emergency procedures:**

Wear protective equipment. Transfer to a disposal or recovery container.Use respiratory protective device against the effects of fumes/dust/aerosol. Keep unprotected persons away. Ensure adequate ventilation.Keep away from ignition sources. Protect from heat.

**Environmental precautions:**

Prevent from reaching drains, sewer or waterway. Collect contaminated soil for characterization per Section 13

**Methods and material for containment and cleaning up:**

If in a laboratory setting, follow Chemical Hygiene Plan procedures.Place into properly labeled containers for recovery or disposal. If necessary, use trained response staff/contractor.Collect liquid and dilute with water. Neutralize with dilute acid solutions. Decant water to drain with excess water. Absorb with suitable material. Dispose of remaining solid as normal refuse. Always obey local regulations.

**Reference to other sections:**

## SECTION 7 : Handling and storage

**Precautions for safe handling:**

Absorb spillage to prevent material damage due to corrosiveness to metal. Avoid contact with eyes, skin, and clothing. Wash hands after handling. Do not mix with acids.Follow good hygiene procedures when handling chemical materials. Use only in well ventilated areas.

**Conditions for safe storage, including any incompatibilities:**

Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store away from foodstuffs. Store away from oxidizing agents.Store in cool, dry conditions in well sealed containers. Store with Corrosives.

## SECTION 8 : Exposure controls/personal protection

| | |
|---|---|
| **Control Parameters:** | 1310-73-2, Sodium Hydroxide, OSHA PEL TWA 2 mg/m3<br>1310-73-2, Sodium Hydroxide, ACGIH TLV TWA 2 mg/m3 |
| **Appropriate Engineering controls:** | Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use/handling.Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapor or dusts (total/respirable) below the applicable workplace exposure limits (Occupational Exposure Limits-OELs) indicated above.Use under a chemical fume hood. |
| **Respiratory protection:** | Use suitable respiratory protective device when high concentrations are present. Use suitable respiratory protective device when aerosol or mist is formed. For spills, respiratory protection may be advisable. Use under a chemical fume hood. |
| **Protection of skin:** | The glove material has to be impermeable and resistant to the product/ the substance/ the preparation being used/handled.Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation. |
| **Eye protection:** | Safety glasses with side shields or goggles. |
| **General hygienic measures:** | The usual precautionary measures are to be adhered to when handling chemicals. Keep away from food, beverages and feed sources. Immediately remove all soiled and contaminated clothing. Wash hands before breaks and at the end of work. Do not inhale gases/fumes/dust/mist/vapor/aerosols. Avoid contact with the eyes and skin. |

## SECTION 9 : Physical and chemical properties

| | | | |
|---|---|---|---|
| **Appearance (physical state,color)**: | Clear, colorless liquid | **Explosion limit lower**:<br>**Explosion limit upper**: | Non Explosive<br>Non Explosive |
| **Odor**: | Odorless | **Vapor pressure**: | 14mmHg @ 20C |
| **Odor threshold**: | Not Determined | **Vapor density**: | >1 |
| **pH-value**: | 13.3 | **Relative density**: | Approx 1 |
| **Melting/Freezing point**: | Approx 0°C | **Solubilities**: | Soluble in Water |
| **Boiling point/Boiling range**: | Approx 100°C | **Partition coefficient (n-octanol/water)**: | Not Determined |
| **Flash point (closed cup)**: | Not Determined | **Auto/Self-ignition temperature**: | Not Determined |
| **Evaporation rate**: | Not Determined | **Decomposition temperature**: | Not Determined |
| **Flammability (solid,gaseous)**: | Not Determined | **Viscosity**: | a. Kinematic:Not Determined<br>b. Dynamic: Not Determined |
| **Density**: Not Determined | | | |

## SECTION 10 : Stability and reactivity

**Reactivity:** solution attacks metals such as aluminium, tin, lead and zinc Also generates heat on exposure to acids. Aqueous solutions react violently with acids.
**Chemical stability:**No decomposition if used and stored according to specifications.
**Possible hazardous reactions:**

**Conditions to avoid:**Incompatible materials, excess heat
**Incompatible materials:**acids, Organic materials, Chlorinated solvents, Aluminum, Phosphorus, Tin/tin oxides, Zinc
**Hazardous decomposition products:**sodium oxides, hydrogen

## SECTION 11 : Toxicological information

| | | |
|---|---|---|
| **Acute Toxicity**: No additional information. | | |
| **Chronic Toxicity**: No additional information. | | |
| **Corrosion Irritation**: | | |
| **Ocular**: | 1310-73-2 | Rabbit: Corrosive to eyes |
| **Dermal**: | 1310-73-2 | Rabbit: Causes Burns |
| **Sensitization**: | | No additional information. |
| **Single Target Organ (STOT)**: | | No additional information. |
| **Numerical Measures**: | | No additional information. |
| **Carcinogenicity**: | | Not listed as a carcinogen: 1310-73-2 |
| **Mutagenicity**: | | No additional information. |
| **Reproductive Toxicity**: | | No additional information. |

## SECTION 12 : Ecological information

**Ecotoxicity Persistence and degradability**: Readily degradable in the environment.
**Bioaccumulative potential**: Not Bioaccumulative.
**Mobility in soil**: -1.87 (water)
**Other adverse effects**:

## SECTION 13 : Disposal considerations

**Waste disposal recommendations**:

Product/containers must not be disposed together with household garbage. Do not allow product to reach sewage system or open water.It is the responsibility of the waste generator to properly characterize all waste materials according to applicable regulatory entities (US 40CFR262.11). Consult federal state/ provincial and local regulations regarding the proper disposal of waste material that may incorporate some amount of this product.Neutralize with dilute acid solutions.

## SECTION 14 : Transport information

**UN-Number**

1824

**UN proper shipping name**

Sodium hydroxide solution

**Transport hazard class(es)**

**Class:**
8 Corrosive substances

**Packing group**:II
**Environmental hazard**:
**Transport in bulk**:
**Special precautions for user**:

## SECTION 15 : Regulatory information

**United States (USA)**

**SARA Section 311/312 (Specific toxic chemical listings)**:

None of the ingredients is listed

**SARA Section 313 (Specific toxic chemical listings)**:

None of the ingredients is listed

**RCRA (hazardous waste code)**:

None of the ingredients is listed

**TSCA (Toxic Substances Control Act)**:

All ingredients are listed.

**CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act)**:

1310-73-2 Sodium Hydroxide 1000 lb

**Proposition 65 (California)**:

**Chemicals known to cause cancer**:

None of the ingredients is listed

**Chemicals known to cause reproductive toxicity for females**:

None of the ingredients is listed

**Chemicals known to cause reproductive toxicity for males**:

None of the ingredients is listed

**Chemicals known to cause developmental toxicity**:

None of the ingredients is listed

**Canada**

**Canadian Domestic Substances List (DSL)**:

All ingredients are listed.

**Canadian NPRI Ingredient Disclosure list (limit 0.1%)**:

None of the ingredients is listed

**Canadian NPRI Ingredient Disclosure list (limit 1%)**:

1310-73-2 Sodium Hydroxide

## SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the SDS contains all the information required by the Controlled Products Regulations.Note:. The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this

material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.
**GHS Full Text Phrases**:

**Abbreviations and acronyms**:
IMDG: International Maritime Code for Dangerous Goods
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)

**Effective date** : 12.14.2014
**Last updated** : 03.25.2015

# Safety Data Sheet

## SECTION 1: Identification

### 1.1. Product Identifier

**Trade Name or Designation:** Sodium Phosphate Dibasic Heptahydrate, ACS Reagent Grade
(Disodium Hydrogen Phosphate Heptahydrate)

**Product Number:** 1-32400

**Other Identifying Product Numbers:** 1-32400-1, 1-32400-3, 1-32400-5

### 1.2. Recommended Use and Restrictions on Use

General Laboratory Reagent

### 1.3. Details of the Supplier of the Safety Data Sheet

**Company:** Reagents Inc.

**Address:** 4746 Sweden Road
Charlotte, NC 28224 USA

**Telephone:** 800-732-8484

### 1.4. Emergency Telephone Number (24 hr)

CHEMTREC (USA) 800-424-9300
CHEMTREC (International) 1+ 703-527-3887

## SECTION 2: Hazard(s) Identification

### 2.1. Classification of the Substance or Mixture (in accordance with OSHA HCS 29 CFR 1910.1200)

*For the full text of the Hazard and Precautionary Statements listed below, see Section 16.*

This product is not categorized as hazardous in any GHS hazard class.

### 2.2. GHS Label Elements

**Pictograms:** None required.

**Signal Word:** None required.

**Hazard Statements:** None required.

**Precautionary Statements:** None required.

### 2.3. WHMIS Classification

WHMIS classification is not included based on the recommended option (Option 4) found in the Canada Gazette Part II, Vol. 149, No.3, page 458

### 2.4. Hazards not Otherwise Classified or Covered by GHS

Data not available.

## SECTION 3: Composition / Information on Ingredients

### 3.1. Components of Substance or Mixture

| Chemical Name | Formula | Molecular Weight | CAS Number | Weight% |
|---|---|---|---|---|
| Sodium Phosphate, Dibasic, Heptahydrate | $Na_2HPO_4 \cdot 7H_2O$ | Data not available. | 7782-85-6 | 100.00% |

## SECTION 4: First-Aid Measures

### 4.1. General First Aid Information

**Eye Contact:** May cause irritation, redness, pain, and tearing.

**Inhalation:** Not expected to require first aid. If necessary, remove to fresh air.

**Skin Contact:** May cause slight irritation to those allergic to phosphates.

**Ingestion:** Dilute with water or milk. Do not induce vomiting. Call a physician if necessary.

### 4.2. Most Important Symptoms and Effects, Acute and Delayed

May cause irritation to the eyes and skin. Wash areas of contact with water. Call a physician if irritation develops. Does not present any significant health hazards. May be harmful if swallowed or inhaled.

EYE CONTACT: May cause irritation, redness, pain, and tearing. SKIN CONTACT: May cause slight irritation to those allergic to phosphates. CHRONIC EFFECTS / CARCINOGENICITY: May sequester calcium and cause calcium phosphate deposits in the kidneys.

### 4.3. Medical Attention or Special Treatment Needed

Not expected to require special treatment.

## SECTION 5: Fire-Fighting Measures

### 5.1. Extinguishing Media

Use any means suitable for extinguishing surrounding fire.

### 5.2. Specific Hazards Arising from the Substance or Mixture

Not considered to be a fire or explosion hazard.

### 5.3. Special Protective Equipment for Firefighters

Use protective clothing and breathing equipment appropriate for the surrounding fire.

# Safety Data Sheet

## SECTION 6: Accidental Release Measures

### 6.1. Personal Precautions, Protective Equipment and Emergency Procedures

Wear appropriate PPE for the size and nature of the spill. As a general rule, wear safety glasses and gloves.

### 6.2. Cleanup and Containment Methods and Materials

Pick up in a manner that does not generate dust. Powder may be moistened with water to aid in the clean-up.

## SECTION 7: Handling and Storage

### 7.1. Precautions for Safe Handling and Storage Conditions

As with all chemicals, wash hands thoroughly after handling. Avoid contact with eyes and skin. Protect from physical damage. Keep in tightly closed containers in a cool, dry area.

## SECTION 8: Exposure Controls / Personal Protection

### 8.2. Exposure Controls

**Engineering Controls:** No specific controls are needed. Normal room ventilation is adequate.

**Respiratory Protection:** Normal room ventilation is adequate.

**Skin Protection:** Chemical resistant gloves.

**Eye Protection:** Safety glasses or goggles.

### 8.3. Personal Protective Equipment

Normal room ventilation is adequate. Chemical resistant gloves. Safety glasses or goggles.

# Safety Data Sheet

## SECTION 9: Physical and Chemical Properties

### 9.1. Basic Physical and Chemical Properties

| | |
|---|---|
| **Appearance:** | Colorless/white solid |
| **Physical State:** | Solid |
| **Odor:** | Data not available. |
| **Odor Threshold:** | Data not available. |
| **pH:** | Data not available. |
| **Melting/Freezing Point:** | Data not available. |
| **Initial Boiling Point /Range:** | Data not available. |
| **Flash Point:** | Data not available. |
| **Evaporation Rate:** | Data not available. |
| **Flammability:** | Data not available. |
| **Flammability/Explosive Limits:** | Data not available. |
| **Vapor Pressure:** | Data not available. |
| **Vapor Density:** | Data not available. |
| **Relative Density:** | 1.70 |
| **Solubility:** | Data not available. |
| **Partition Coeffieient (n-Octanol/Water):** | Data not available. |
| **Auto-Ignition Temperature:** | Data not available. |
| **Decomposition Temperature:** | Data not available. |
| **Viscosity:** | Data not available. |
| **ExplosiveProperties:** | Data not available. |
| **Oxidizing Properties:** | Data not available. |

## SECTION 10: Stability and Reactivity

### 10.1. Reactivity and Chemical Stability

Stable under normal conditions of use and storage.

### 10.2. Possibility of Hazardous Reactions

Data not available.

### 10.3. Conditions to Avoid and Incompatible Materials

Reactive with acids, alkalis.

### 10.4. Hazardous Decomposition Products

Will not occur.

# Safety Data Sheet

## SECTION 11: Toxicological Information

### 11.1. Information on Toxicological Effects

**Acute Toxicity - Oral Exposure:**

Not applicable.

**Acute Toxicity - Dermal Exposure:**

Not applicable.

**Acute Toxicity - Inhalation Exposure:**

Not applicable.

**Acute Toxicity - Other Information:**

LD50, Oral, Rat: (Sodium Phosphate, Dibasic, Heptahydrate) 12.93 g/kg, details of toxic effects not reported other than lethal dose value.

**Skin Corrosion and Irritation:**

Not applicable.

**Serious Eye Damage and Irritation:**

Not applicable.

**Respiratory Sensitization:**

Not applicable.

**Skin Sensitization:**

Not applicable.

**Germ Cell Mutagenicity:**

Not applicable.

**Carcinogenicity:**

Not applicable.

**Reproductive Toxicity:**

Not applicable.

**Specific Target Organ Toxicity from Single Exposure:**

Not applicable.

**Specific Target Organ Toxicity from Repeated Exposure:**

Not applicable.

**Aspiration Hazard:**

Not applicable.

**Additional Toxicology Information:**

Data not available.

# Safety Data Sheet

## SECTION 12: Ecological Information

### 12.1. Ecotoxicity

Not applicable.

### 12.2. Persistence and Degradability

Data not available.

### 12.3. Bioaccumulative Potential

Data not available.

### 12.4. Mobility in Soil

Data not available.

### 12.5. Other Adverse Ecological Effects

Data not available.

## SECTION 13: Disposal Considerations

### 13.1. Waste Treatment Methods

Data not available.

## SECTION 14: Transportation Information

### 14.1. Transportation by Land - Department of Transportation (DOT, United States of America)

Not regulated according to DOT Regulations.

# Safety Data Sheet

### 14.2. Transportation by Air - International Air Transport Association (IATA)

**UN Number:** N/A

**Proper Shipping Name:**

**Hazard Class:**

**Packing Group:**

**Hazard Placard Labels:**

## SECTION 15: Regulatory Information

### 15.1. Occupational Safety and Health Administration (OSHA) Hazards

Not listed.

### 15.2. Superfund Amendments and Reauthorization Act (SARA) 302 Extremely Hazardous Substances

Not listed.

### 15.3. Superfund Amendments and Reauthorization Act (SARA) 311/312 Hazardous Chemicals

Sodium Phosphate, Dibasic, Heptahydrate (CAS # 7782-85-6): 5000 lb final RQ; 2270 kg final RQ

### 15.4. Superfund Amendments and Reauthorization Act (SARA) 313 Toxic Release Inventory (TRI)

Not listed.

### 15.5. Massachusetts Right-to-Know Substance List

Sodium Phosphate, Dibasic, Heptahydrate (CAS # 7782-85-6): Present

### 15.6. Pennsylvania Right-to-Know Hazardous Substances

Sodium Phosphate, Dibasic, Heptahydrate (CAS # 7782-85-6): Environmental hazard

Sodium Phosphate, Dibasic, Heptahydrate (CAS # 7782-85-6): Present

### 15.7. New Jersey Worker and Community Right-to-Know Components

Sodium Phosphate, Dibasic, Heptahydrate (CAS # 7782-85-6): sn 1723

### 15.8. California Proposition 65

Not listed.

### 15.9. Canada Domestic Substances List / Non-Domestic Substances List (DSL/NDSL)

Sodium Phosphate, Dibasic, Heptahydrate (CAS # 7782-85-6): Present

### 15.10. United States of America Toxic Substances Control Act (TSCA) List

Sodium Phosphate, Dibasic, Heptahydrate (CAS # 7782-85-6): Present

# Safety Data Sheet

### 15.11. European Inventory of Existing Commercial Chemical Substances (EINECS), European List of Notified Chemical Substances (ELINCS), and No Longer Polymers (NLP)

Not listed.

## SECTION 16: Other Information

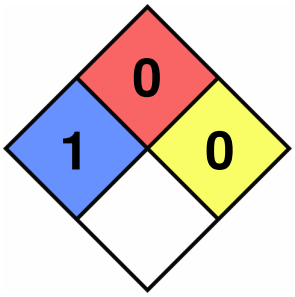
### 16.1. Full Text of Hazard Statements and Precautionary Statements

### 16.2. Miscellaneous Hazard Classes

**Canadian Carcinogenicity Hazard Class:** Not Applicable.
**Physical Hazards Not Otherwise Classified (PHNOC):** Not Applicable.
**Health Hazards Not Otherwise Classified (HHNOC):** Not Applicable.
**Biohazardous Infectious Materials Hazard Class:** Not Applicable.

### 16.3. National Fire Protection Association (NFPA) Rating

**Health:** 1
**Flammability:** 0
**Reactivity:** 0
**Special Hazard:**




### 16.4. Document Revision

**Last Revision Date:** 8/31/2015

## DISCLAIMER

When handled properly by qualified personnel, the product described herein does not present a significant health or safety hazard. Alteration of its characteristics by concentration, evaporation, addition of other substances, or other means may present hazards not specifically addressed herein and which must be evaluated by the user. The information furnished herein is believed to be accurate and represents the best data currently available to us. No warranty, expressed or implied, is made and REAGENTS, INC. assumes no legal responsibility or liability whatsoever resulting from its use.

Date of Issue:
19 February 2019

# SAFETY DATA SHEET

## 1. SUBSTANCE AND SOURCE IDENTIFICATION

**Product Identifier**

**SRM Number:** 723e
**SRM Name:** Tris(hydroxymethyl)aminomethane $(HOCH_2)_3CNH_2$ Acidimetric Standard
**Other Means of Identification:** Not applicable.

**Recommended Use of This Material and Restrictions of Use**

This Standard Reference Material (SRM) consists of highly purified tris(hydroxymethyl)aminomethane $(HOCH_2)_3CNH_2$ [2-amino-2-(hydroxymethyl)-1,3-propanediol; "Tris"; "THAM"], hereafter referred to as Tris. SRM 723e is intended primarily for use in acidimetric standardization. A unit of SRM 723e consists of 50 g in a clear glass bottle.

**Company Information**

National Institute of Standards and Technology
Standard Reference Materials Program
100 Bureau Drive, Stop 2300
Gaithersburg, Maryland 20899-2300

Telephone: 301-975-2200
FAX: 301-948-3730
E-mail: SRMMSDS@nist.gov
Website: https://www.nist.gov/srm

Emergency Telephone ChemTrec:
1-800-424-9300 (North America)
+1-703-527-3887 (International)

## 2. HAZARDS IDENTIFICATION

**Classification**

| | | |
|---|---|---|
| **Physical Hazard:** | Not classified. | |
| **Health Hazard:** | Skin Corrosion/Irritation | Category 2 |
| | Serious Eye damage/Eye irritation | Category 2A |
| | STOT - Single Exposure | Category 3 |

**Label Elements**

**Symbol**

**Signal Word**
Warning

**Hazard Statement(s):**

| | |
|---|---|
| H315 | Causes skin irritation. |
| H319 | Causes serious eye irritation. |
| H335 | May cause respiratory irritation. |

**Precautionary Statement(s):**

| | |
|---|---|
| P261 | Avoid breathing dust. |
| P264 | Wash hands thoroughly after handling. |
| P271 | Use only outdoors or in a well-ventilated area. |
| P280 | Wear protective gloves. |
| P302+P352 | If on skin: Wash with plenty of water. |
| P304+P340 | If inhaled: Remove person to fresh air and keep comfortable for breathing. |
| P305+P351+P338 | If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| P322+P364 | Take off contaminated clothing and wash it before reuse. |

| | |
|---|---|
| P332+P337+P313 | If skin or eye irritation occurs: Get medical attention. |
| P403+P233 | Store in a well-ventilated place. Keep container tightly closed. |
| P405 | Store locked up. |
| P501 | Dispose of contents and containers in accordance with all applicable regulations. |

**Hazards Not Otherwise Classified:** Not applicable.

**Ingredients(s) with Unknown Acute Toxicity:** Not applicable.

## 3. COMPOSITION AND INFORMATION ON HAZARDOUS INGREDIENTS

**Substance:** Tris(hydroxymethyl)aminomethane

**Other Designations:** Tris [tris buffering agent; trishydroxymentylaminomethane; tris(methylolamino)methane; 1,1,1 tris(hydroxymethyl)methanamine; tris(hydroxymethyl)methylamine; tris(hydroxymethyl)methanamine]

Components are listed in compliance with OSHA's 29 CFR 1910.1200; for the actual values see the Certificate of Analysis.

| Hazardous Component(s) | CAS Number | EC Number (EINECS) | Nominal Mass Concentration (%) |
|---|---|---|---|
| Tris(hydroxymethyl)aminomethane | 77-86-1 | 201-064-4 | 100 |

## 4. FIRST AID MEASURES

**Description of First Aid Measures:**

**Inhalation:** If adverse effects occur, remove to uncontaminated area. If not breathing, give artificial respiration or oxygen by qualified personnel. Seek immediate medical attention.

**Skin Contact:** Wash skin with soap and water for at least 15 minutes. Thoroughly clean and dry contaminated clothing before reuse.

**Eye Contact:** Flush eyes with water for at least 15 minutes. If necessary, seek medical attention.

**Ingestion:** If a large amount is swallowed, get medical attention.

**Most Important Symptoms/Effects, Acute and Delayed:** Skin, eye, and possible respiratory irritation.

**Indication of any immediate medical attention and special treatment needed, if necessary:** If any of the above symptoms are present, seek medical attention if needed.

## 5. FIRE FIGHTING MEASURES

**Fire and Explosion Hazards:** Negligible fire hazard. See Section 9, "Physical and Chemical Properties" for flammability properties.

**Extinguishing Media:**

Suitable: Regular dry chemical, dry sand, water, and regular foam.
Unsuitable: None listed.

**Specific Hazards Arising from the Chemical:** None listed.

**Special Protective Equipment and Precautions for Fire-Fighters:** Avoid inhalation of material or combustion byproducts. Wear full protective clothing and NIOSH approved self-contained breathing apparatus (SCBA).

**NFPA Ratings** (0 = Minimal; 1 = Slight; 2 = Moderate; 3 = Serious; 4 = Severe)

Health = 2 Fire = 0 Reactivity = 0

## 6. ACCIDENTAL RELEASE MEASURES

**Personal Precautions, Protective Equipment and Emergency Procedures:** Use suitable protective equipment; see Section 8, "Exposure Controls and Personal Protection".

**Methods and Materials for Containment and Clean up:** Keep out of water supplies and sewers. Do not touch spilled material. Notify safety personnel of spills. Collect spilled material in appropriate container for disposal. Isolate hazard area and deny entry.

## 7. HANDLING AND STORAGE

**Safe Handling Precautions:** Minimize dust generation. See Section 8, "Exposure Controls and Personal Protection".

**Storage:** Store and handling in accordance with all current regulations and standards. Keep separated from incompatible substances (acids, bases, metals, oxidizing materials).

## 8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

**Exposure Limits:** No occupational exposure limits have been established. The exposure limits for Particulates Not Otherwise Regulated are applicable.

OSHA (PEL): 15 mg/m$^3$ (TWA, total particulates)
5 mg/m$^3$ (TWA, respirable particulates)

**Engineering Controls:** Provide local exhaust or process enclosure ventilation system. Ensure compliance with applicable exposure limits.

**Personal Protection:** In accordance with OSHA 29 CFR 1910.132, subpart I, wear appropriate Personal Protective Equipment (PPE) to minimize exposure to this material.

**Respiratory Protection:** If workplace conditions warrant a respirator, a respiratory protection program that meets OSHA 29CFR 1910.134 must be followed. Refer to NIOSH 42 CFR 84 for applicable certified respirators.

**Eye/Face Protection:** Wear splash resistant safety goggles with a face shield. An eye wash station should be readily available near areas of use.

**Skin and Body Protection:** Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product. Chemical-resistant gloves should be worn at all times when handling chemicals.

## 9. PHYSICAL AND CHEMICAL PROPERTIES

| | |
|---|---|
| **Descriptive Properties:** | |
| **Appearance (physical state, color, etc.):** | white crystalline, hygroscopic powder |
| **Molecular Formula:** | $C_4H_{11}NO_3$ |
| **Molar Mass (g/mol):** | 121.1 |
| **Odor:** | odorless |
| **Odor threshold:** | not available |
| **pH (solution):** | 10.4 at 1.2 % |
| **Evaporation rate:** | not applicable |
| **Melting point/freezing point (ºC):** | 171–172 (340–342 ºF) |
| **Relative Density (g/L):** | not available |
| **Vapor Pressure (mmHg):** | not applicable |
| **Vapor Density (air = 1):** | not applicable |
| **Viscosity (cP):** | not applicable |
| **Solubility(ies):** | water soluble (55 %); moderately soluble: methanol, ethanol, ethylene glycol, dimethylformamide; slightly soluble: acetone, ether; very slightly soluble: ethyl acetate, cyclohexane, chloroform, carbon tetrachloride |
| **Partition coefficient (n-octanol/water):** | log Kow = –1.56 |
| **Particle Size (if relevant)** | not available |
| **Thermal Stability Properties:** | |
| **Autoignition Temperature (ºC):** | not available |
| **Thermal Decomposition (ºC):** | not available |
| **Initial boiling point and boiling range (ºC):** | 219–220 (426–428 ºF) at 10 mmHg |
| **Explosive Limits, LEL (Volume %):** | not available |
| **Explosive Limits, UEL (Volume %):** | not available |
| **Flash Point (ºC)** | not available |
| **Flammability (solid, gas):** | not available |

## 10. STABILITY AND REACTIVITY

**Reactivity:** Stable at normal temperatures and pressure.

**Stability:** X Stable _____ Unstable

**Possible Hazardous Reactions:** None listed.

**Conditions to Avoid:** Avoid generating dust and exposure to moisture.

**Incompatible Materials:** Acids, bases, metals, and oxidizing materials.

**Fire/Explosion Information:** See Section 5, "Fire Fighting Measures".

**Hazardous Decomposition:** Thermal decomposition will produce oxides of nitrogen and carbon.

**Hazardous Polymerization:** _____ Will Occur X Will Not Occur

## 11. TOXICOLOGICAL INFORMATION

**Route of Exposure:** X Inhalation _____ Skin _____ Ingestion

**Symptoms Related to the Physical, Chemical and Toxicological Characteristics:** Skin, eye, and possible respiratory irritation.

**Potential Health Effects (Acute, Chronic and Delayed):**

**Inhalation:** Inhalation may cause irritation of the mucous membranes with tightness and pain in the chest, coughing, and difficulty breathing.

**Skin Contact:** Skin exposure may result in irritation with redness, pain, and possibly sensitization. Dermatitis may develop due to irritation or sensitization over time.

**Eye Contact:** Eye irritation with redness, pain, and possibly corneal damage may occur. Prolonged or repeated exposure may result in conjunctivitis.

**Ingestion:** Ingestion of this material is unlikely under normal conditions of use. If ingested, gastrointestinal irritation and possible burns to the mouth and stomach may result. A large does to laboratory animals caused weakness, collapse, and death.

**Numerical Measures of Toxicity:**

**Acute Toxicity:** Not classified.
Rat, Oral LD50: >3000 mg/kg

**Skin Corrosion/Irritation:** Category 2
Rabbit, Dermal: 25 % moderate; 500 mg severe
Woman, Dermal: 1 % moderate

**Serious Eye damage/Eye irritation:** Category 2A, No data available on effects on the eyes, deemed to be a Category 2A based on skin irritation data.

**Respiratory Sensitization:** Not classified; no data available.

**Skin Sensitization:** Not classified; no data available.

**Germ Cell Mutagenicity:** Not classified; no data available.

**Carcinogenicity:** Not classified.

**Listed as a Carcinogen/Potential Carcinogen** _____ Yes X No
Tris(Hydroxymethyl)Aminomethane is not listed by IARC, NTP or OSHA as a carcinogen.

**Reproductive Toxicity:** Not classified.
Rat, Oral TDLo: 12 000 mg/kg (prior to copulation 14 d, 4 d, continuous)

**Specific Target Organ Toxicity, Single Exposure:** Not classified; no data available.

**Specific Target Organ Toxicity, Repeated Exposure:** Category 3; May cause respiratory irritation of the mucous membranes with tightness and pain in the chest, coughing, and difficulty breathing.

**Aspiration Hazard:** Not classified; no data available.

## 12. ECOLOGICAL INFORMATION

**Ecotoxicity Data:** No data available.

**Persistence and Degradability:** Biodegradation may be slow in the environment.

**Bioaccumulative Potential:** Potential for bioconcentration in aquatic organisms is low (estimated BCF equals 3).

**Mobility in Soil:** Very high mobility in soil.

**Other Adverse effects:** No data available.

## 13. DISPOSAL CONSIDERATIONS

**Waste Disposal:** Dispose of waste in accordance with all applicable federal, state, and local regulations.

## 14. TRANSPORTATION INFORMATION

**U.S. DOT and IATA:** Not regulated by DOT or IATA.

## 15. REGULATORY INFORMATION

**U.S. Regulations:**

CERCLA Sections 102a/103 (40 CFR 302.4): Not regulated.

SARA Title III Section 302 (40 CFR 355.30): Not regulated.

SARA Title III Section 304 (40 CFR 355.40): Not regulated.

SARA Title III Section 313 (40 CFR 372.65): Not regulated.

OSHA Process Safety (29 CFR 1910.119): Not regulated.

SARA Title III Sections 311/312 Hazardous Categories (40 CFR 370.21):

| | |
|---|---|
| ACUTE HEALTH: | Yes. |
| CHRONIC HEALTH: | No. |
| FIRE: | No. |
| REACTIVE: | No. |
| PRESSURE: | No. |

**State Regulations:**

California Proposition 65: Not listed.

**U.S. TSCA Inventory:** Listed.

**TSCA 12(b), Export Notification:** Not listed.

**Canadian Regulations:** WHMIS Information: Not provided for this material.

## 16. OTHER INFORMATION

**Issue Date:** 19 February 2019

**Sources:** ChemAdvisor, Inc., SDS *Tris(Hydroxymethyl)Aminomethane*, 09 December 2015.

Hazardous Substances Data Bank, National Library of Medicine, *Tromethamine* CAS# 77-86-1, Full Record, available at https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (accessed Feb 2019).

Center for Disease Control (CDC), NIOSH Pocket Guide to Chemical Hazards, *Particulates Not Otherwise Regulated*, available at https://www.cdc.gov/niosh/npg/npgd0480.html (accessed Feb 2019).

Sigma-Aldrich, Vendor MSDS *Trizma® base*, 07 November 2017.

**Key of Acronyms:**

| | | | |
|---|---|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists | NIOSH | National Institute for Occupational Safety and Health |
| ALI | Annual Limit on Intake | NIST | National Institute of Standards and Technology |
| CAS | Chemical Abstracts Service | NRC | Nuclear Regulatory Commission |
| CEN | European Committee for Standardization | NTP | National Toxicology Program |
| CERCLA | Comprehensive Environmental Response, Compensation, and Liability Act | OSHA | Occupational Safety and Health Administration |
| CFR | Code of Federal Regulations | PEL | Permissible Exposure Limit |
| CPSU | Coal Mine Dust Personal Sample Unit | RCRA | Resource Conservation and Recovery Act |
| DOT | Department of Transportation | REL | Recommended Exposure Limit |
| EC50 | Effective Concentration, 50 % | RM | Reference Material |
| EINECS | European Inventory of Existing Commercial Chemical Substances | RQ | Reportable Quantity |
| EPCRA | Emergency Planning and Community Right-to-Know Act | RTECS | Registry of Toxic Effects of Chemical Substances |
| IARC | International Agency for Research on Cancer | SARA | Superfund Amendments and Reauthorization Act |
| IATA | International Air Transport Association | SCBA | Self-Contained Breathing Apparatus |
| IDLH | Immediately Dangerous to Life and Health | SRM | Standard Reference Material |
| ISO | International Organization for Standardization | STEL | Short Term Exposure Limit |
| LC50 | Lethal Concentration, 50 % | TDLo | Toxic Dose Low |
| LD50 | Lethal Dose, 50 % | TLV | Threshold Limit Value |
| LEL | Lower Explosive Limit | TPQ | Threshold Planning Quantity |
| MSDS | Material Safety Data Sheet | TSCA | Toxic Substances Control Act |
| NFPA | National Fire Protection Association | TWA | Time Weighted Average |
| MSHA | Mine Safety and Health Administration | UEL | Upper Explosive Limit |
| | | WHMIS | Workplace Hazardous Materials Information System |

**Disclaimer:** Physical and chemical data contained in this SDS are provided only for use in assessing the hazardous nature of the material. The SDS was prepared carefully, using current references; however, NIST does not certify the data in the SDS. The certified values for this material are given in the NIST Certificate of Analysis.

Users of this SRM should ensure that the SDS in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srmmsds@nist.gov; or via the Internet at https://www.nist.gov/srm.

## E. Patents

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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22 February 2007 (22.02.2007)

PCT

(10) International Publication Number
**WO 2007/022425 A2**

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***C07H 21/04*** **(2006.01)** ***C07K 14/11*** **(2006.01)**
***C12N 7/00*** **(2006.01)**

(21) **International Application Number:**
PCT/US2006/032353

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**(25) Filing Language:** English

**(26) Publication Language:** English

**(30) Priority Data:**
60/708,988 16 August 2005 (16.08.2005) US

(71) **Applicant** *(for all designated States except US):* **HAWAII BIOTECH, INC.** [US/US]; 99-193 Aiea Heights Dr., Ste 200, Aiea, HI 96701 (US).

**(72) Inventors; and**
**(75) Inventors/Applicants** *(for US only):* **WEEKS-LEVY, Carolyn** [US/US]; 1045 Kaupaku Place, Honolulu, HI 96825 (US). **CLEMENTS, David,** E. [US/US]; 1322 10th Ave., Honolulu, HI 96816 (US). **OGATA, Steven,** A. [US/US]; 1376 Akiahala Street, Kailua, HI 96734 (US).

**(74) Agent: DARBY, George,** E.; Paradise Patent Services, Inc., P.O. Box 893010, Mililani, HI 96789-0010 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV,LY,MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

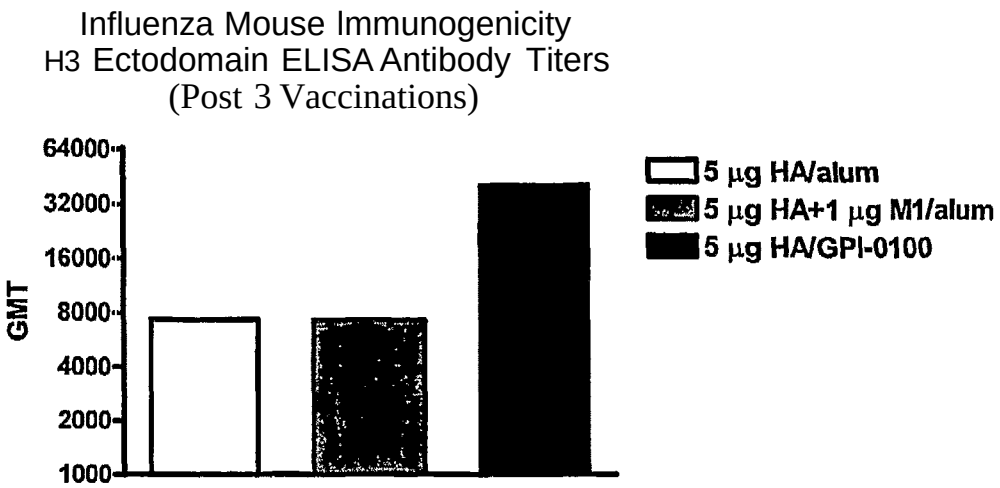
**(84) Designated States** *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, **DK,** EE, ES, FI, FR, GB, GR, HU, IE, **IS, IT,** LT, LU, LV, MC, NL, PL, PT, **RO,** SE, **SI,** SK, TR), OAPI (BF, **BJ,** CF, CG, CI, CM, GA, GN, GQ, GW, ML, **MR,** NE, SN, TD, TG).

**Declarations under Rule** 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(U))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(Ui))*
— *of inventorship (Rule 4.17 (iv))*

**Published:**
— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**(54) Title:** INFLUENZA RECOMBINANT SUBUNIT VACCINE




**(57) Abstract:** The invention provides influenza proteins, including subunit proteins and immunogenic compositions that can be utilized, with or without adjuvants, as vaccines to protect against influenza infection in animal models and humans. The recombinant proteins are expressed from transformed insect cells that contain integrated copies of the appropriate expression cassettes in their genome. The invention uses a Drosophila melanogaster expression system to provide high yields of recombinant subunit proteins with native-like conformation.

WO 2007/022425 A2

# INFLUENZA RECOMBINANT SUBUNIT VACCINE

## RELATED APPLICATION

[01] This application claims the benefit of U.S. Provisional Patent Application No. 60/708,988, filed August 16, 2005, the disclosures and drawings of which prior application are hereby incorporated by reference in their entirety.

## INCORPORATION OF SEQUENCE LISTING

[02] A sequence listing file in ST.25 format on CD-ROM is appended to this application and fully incorporated herein by reference. The sequence listing information recorded in computer readable form is identical to the written sequence listing (per WIPO ST.25 para. 39, the information recorded on the form is identical to the written sequence listing). With respect to the appended CD-ROMs, the format is ISO 9660; the operating system compatibility is MS-Windows; the single file contained on each CD-ROM is named "FLU.S2.ADJ.04.ST25.txt" and is a text file produced by Patentln 3.3 software; the file size in bytes is 35 KB; and the date of file creation is 16 August 2006. The contents of the two CD-ROMs submitted herewith are identical.

## BACKGROUND OF THE INVENTION

### TECHNICAL FIELD

[03] The invention relates to vaccine formulations designed to protect against influenza. In particular, the vaccine formulations comprise recombinant subunit proteins derived from influenza virus, and optionally include one or more adjuvants. "Subunit protein" is defined here as any protein derived or expressed independently from the complete organism that it is derived from. Furthermore, a subunit protein may represent a full length native protein sequence or any fraction of the full length native protein sequence. Additionally, a subunit protein may contain in addition to the full length or partial protein sequence, one or more sequences, which may contain sequences that are homologous or heterologous to the organism from which the primary sequence was derived. This definition is significantly broader than the concept of a subunit protein as a single protein molecule that co-assembles with other protein molecules to form a multimeric or oligomeric protein. The subunit proteins of the invention are produced in a cellular production system by means of recombinant DNA methods and, after purification, are formulated in a vaccine.

immunogen means: 1) much less protein is needed to produce a robust immune response, 2) the quality of the immune response is increased, and 3) the efficacy of subunit vaccines is improved.

**[032]** There is a clear need for new technologies that can be used to respond quickly to influenza outbreaks and pandemics, to produce sufficient doses of high quality and safe vaccine for all populations (including the iromunodef ïcient population), and to produce improved vaccine formulations with increased immunogenicity and efficacy. Some of the technical problems to be solved are engineering nucleotide sequences for immunogenic and protective epitopes, expression and purification of the subunit proteins encoded by the nucleotide sequences through methods that can be scaled up to commercial production, and determining which adjuvants, if any, should be included in vaccine formulations containing the subunit proteins. The invention disclosed herein meets the need of developing a new influenza vaccine production method and solves associated technical problems.

## SUMMARY OF THE INVENTION

**[033]** The invention provides recombinant influenza subunit proteins and immunogenic compositions that can be utilized as vaccines to afford protection against influenza in animal models and humans. The recombinant subunit proteins of the invention are expressed from stably transformed insect cells that contain integrated copies of the appropriate expression cassettes in their genome. The insect cell expression system provides high yields of recombinant subunit proteins with native-like conformation. The recombinant subunit proteins of the invention represent full length or truncated forms of the native influenza proteins. Additionally, multimeric forms of several of the recombinant subunit proteins have been produced. Specifically, the subunits are derived from the HA and M1 proteins of influenza. More specifically the subunit proteins are secreted from the transformed insect cells and then purified from the culture medium following the removal of the host cells. Avoiding lysis of the host cells by either viral means or by physical means simplifies purification, improves yields, and avoids potential degradation of the target protein.

**[034]** The invention also provides for the use of adjuvants as components in an immunogenic composition compatible with the purified proteins to boost the immune response resulting from vaccination. One or more preferred adjuvants are selected from the group comprising saponins (e.g, GP-OlOO), or derivatives thereof, emulsions alone or in combination with carbohydrates or saponins, and aluminum-based adjuvants (collectively, "alum" or "alum-based adjuvants") such as aluminum hydroxide, aluminum phosphate, or a mixture thereof. Aluminum hydroxide (commercially available as "Alhydrogel") was used as alum in the Examples. A

saponin is any plant glycoside with soapy action that can be digested to yield a sugar and a sapogenin aglycone. Sapogenin is the nonsugar portion of a saponin. It is usually obtained by hydrolysis, and it has either a complex terpenoid or a steroid structure that forms a practicable starting point in the synthesis of steroid hormones. The saponins of the invention can be any saponin as described above or saponin-like derivative with hydrophobic regions, especially the strongly polar saponins, primarily the polar triterpensaponins such as the polar acidic bisdesmosides, *e.g.* saponin extract from Quillsjabark Araloside A, Chikosetsusaponin IV, Calendula-Glycoside C, chikosetsusaponin V, Achyranthes-Saponin B. Calendula-Glycoside A, Araloside B, Araloside C, Putranjia-Saponin III, Bersamasaponiside, Putrajia-Saponin IV, Trichoside A, Trichoside B, Saponaside A, Trichoside C, Gypsoside. Nutanoside, Dianthoside C, Saponaside D, aescine from Aesculus hippocastanum or sapoalbin from *Gyposophilla struthium*, preferably, saponin extract *Quillaja saponaria* Molina and Quil A. In addition, saponin may include glycosylated triterpenoid saponins derived from *Quillaja Saponaria* Molina of Beta Amytin type with 8-1 1 carbohydrate moieties as described in U.S. Patent No. 5,679,354. Saponins as defined herein include saponins that may be combined with other materials, such as in an immune stimulating complex ("ISCOM")-like structure as described in U.S. Patent No. 5,679,354. Saponins also include saponin-like molecules derived from any of the above structures, such as GPI-0100, such as described in U.S. Patent No. 6,262,029. Preferably, the saponins of the invention are amphiphilic natural products derived from the bark of the tree, *Quillaia saponaria.* Preferably, they consist of mixtures of triterpene glycosides with an average molecular weight (Mw) of 2000. A particularly preferred embodiment of the invention is a purified fraction of this mixture.

**[035]** The invention further provides methods for utilizing the vaccines to elicit the production of antibodies against the various types and subtypes of influenza virus in a mammalian host as a means of conferring protection against influenza. The vaccine formulations are shown to induce strong overall antibody titers, as well as strong hemagglutinin-inhibition antibody titers, in comparison to other formulations. Furthermore, the vaccine formulations are shown to provide protection against influenza challenge in a mouse model. In comparison to conventionally produced influenza immunogens, the proteins produced by the invention have increased immunogenicity and efficacy, are less costly to produce, and have a shorter production cycle.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[036] FIG. 1.** Lymphocyte proliferation of antigen stimulated splenocytes

**[037] FIG. 2.** IFN-γ production from antigen stimulated splenocytes.

We claim:

1. A method for producing a recombinant subunit influenza vaccine comprising:

expressing and secreting a recombinant influenza hemagglutinin ectodomain protein subunit, wherein the protein subunit lacks a C-terminal transmembrane anchor and is secreted as a soluble protein from stably transformed insect cells; and

formulating said recombinant protein subunit to produce an immunogenic composition that induces the production of hemagglutinin antibody titers in a host vaccinated with the immunogenic composition.

2. A method for producing a recombinant subunit influenza vaccine comprising:

expressing and secreting a recombinant influenza hemagglutinin head protein subunit, wherein the protein subunit lacks a C-terminal transmembrane anchor, lacks an N-terminal portion, and is secreted as a soluble protein from stably transformed insect cells; and

formulating said recombinant protein subunit to produce an immunogenic composition that induces the production of hemagglutinin antibody titers in a host vaccinated with the immunogenic composition.

3. A method for producing a recombinant subunit influenza vaccine comprising:

expressing and secreting a recombinant influenza hemagglutinin ectodomain protein subunit, wherein the protein subunit lacks a C-terminal transmembrane anchor and is secreted as a soluble protein from stably transformed insect cells;

expressing and secreting a recombinant influenza matrix 1 protein subunit, wherein the protein subunit lacks a C-terminal transmembrane anchor, and is secreted as a soluble tetrameric protein from stably transformed insect cells; and

formulating said recombinant hemagglutinin ectodomain and matrix 1 protein subunits to produce an immunogenic composition that induces the production of hemagglutinin antibody titers in a host vaccinated with the immunogenic composition.

4. A method for producing a recombinant subunit influenza vaccine comprising:

expressing and secreting a recombinant influenza hemagglutinin head protein subunit, wherein the protein subunit lacks a C-terminal transmembrane anchor, lacks an N-terminal portion, and is secreted as a soluble protein from stably transformed insect cells;

expressing and secreting a recombinant influenza matrix 1 protein subunit, wherein the protein subunit lacks a C-terminal transmembrane anchor, and is secreted as a soluble tetrameric protein from stably transformed insect cells; and

formulating said recombinant hemagglutinin head and matrix 1 protein subunits to

provide an influenza virus composition that induces the production of Hemagglutinin antibody titers in a host vaccinated with the immunogenic composition.

5. A method for producing a recombinant subunit influenza vaccine comprising:

expressing and secreting a recombinant influenza HA-foldon subunit, wherein the protein is secreted as a soluble protein from stably transformed insect cells; and

formulating said recombinant protein subunit to produce an immunogenic composition that induces the production of hemagglutinin antibody titers in a host vaccinated with the immunogenic composition.

6. A method for producing a recombinant subunit influenza vaccine comprising:

expressing and secreting a recombinant influenza HA-foldon subunit, wherein the protein is secreted as a soluble protein from stably transformed insect cells;

expressing and secreting a recombinant influenza matrix 1 protein subunit, wherein the protein subunit lacks a C-terminal transmembrane anchor, and is secreted as a soluble tetrameric protein from stably transformed insect cells; and

formulating said recombinant HA-foldon and matrix 1 protein subunits to produce an immunogenic composition that induces the production of hemagglutinin antibody titers in a host vaccinated with the immunogenic composition.

7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the influenza virus is influenza A virus.

8. The method of claim 1, 2, 3, 4, 5, or 6, wherein the strain of influenza virus is selected from the group consisting of H5 and H3.

9. The method of claim 1, 2, 3, 4, 5, or 6, wherein the carboxy-teraiinal portion of the hemagglutinin protein subunit is truncated within 10% of the length of a nominal ectodomain.

10. The method of claim 1, 2, 3, 4, 5, or 6, wherein the stably transformed insect cells are *Drosophila melanogaster* S2 cells.

11. The method of claim 1, 2, 3, 4, 5, or 6, wherein formulating the immunogenic composition further comprises including in the immunogenic composition one or more adjuvants.

12. The method of claim 1, 2, 3, 4, 5, or 6, wherein formulating the immunogenic composition further comprises including in the immunogenic composition one or more adjuvants selected from the group consisting of saponin and alum.

13. The method of claim 1, 2, 3, 4, 5, or 6, wherein formulating the immunogenic composition further comprises including in the immunogenic composition GPI-0100 adjuvant.

14. The method of claim 1, 2, 3, 4, 5, or 6, wherein formulating the immunogenic composition further comprises including a pharmaceutically acceptable excipient in the immunogenic composition.

15. The method of claim 1, 2, 3, 4, 5, or 6, wherein the protein subunits are purified by immuno-affinity chromatography.

16. The method of claim 1 or 3, wherein the recombinant influenza hemagglutinin ectodomain protein subunit has an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

17. The method of claim 2 or 4, wherein the recombinant influenza hemagglutinin head protein subunit has an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

18. The method of claim 2 or 4, wherein the truncation points of the hemagglutinin head protein subunit are selected from the group consisting of N-terminal, C-terminal, and N-terminal and C-terminal, wherein the one or both terminal points can be varied up to 10% of the length of a nominal HA-head.

19. The method of claim 3, 4, or 6, wherein the recombinant influenza matrix 1 protein subunit has an amino acid sequence of SEQ ID NO: 10.

20. The method of claim 5 or 6, wherein the recombinant influenza HA-foldon subunit has an amino acid sequence selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO:9.

21. A method for raising an immunogenic response from a subject, comprising administering in a therapeutically acceptable manner a therapeutically effective amount of the immunogenic composition of claim 1, 2, 3, 4, 5, or *6*, to said subject.

22. An immunogenic composition comprising a recombinant subunit influenza vaccine comprising a recombinant influenza hemagglutinin ectodomain protein subunit, wherein the protein subunit lacks a C-terminal transmembrane anchor and is expressed and secreted as a soluble protein from stably transformed insect cells.

23. An immunogenic composition comprising a recombinant subunit influenza vaccine comprising a recombinant influenza hemagglutinin head protein subunit, wherein the hemagglutinin head protein subunit lacks a C-terminal transmembrane anchor, lacks an N-terminal portion, and is expressed and secreted as a soluble protein from stably transformed insect cells.

24. An immunogenic composition comprising a recombinant subunit influenza vaccine comprising a recombinant influenza hemagglutinin ectodomain protein subunit, wherein the hemagglutinin ectodomain protein subunit lacks a C-terminal transmembrane anchor and is expressed and secreted as a soluble protein from stably transformed insect cells,

combined with a recombinant influenza matrix 1 protein subunit, wherein the matrix 1 protein subunit lacks a C-terminal transmembrane anchor, and is expressed and secreted as a soluble tetrameric protein from stably transformed insect cells.

25. An immunogenic composition comprising a recombinant subunit influenza vaccine comprising a recombinant influenza hemagglutinin head protein subunit, wherein the hemagglutinin head protein subunit lacks a C-terminal transmembrane anchor, lacks an N-terminal portion, and is expressed and secreted as a soluble protein from stably transformed insect cells,

combined with a recombinant influenza matrix 1 protein subunit, wherein the matrix 1 protein subunit lacks a C-terminal transmembrane anchor, and is expressed and secreted as a soluble tetrameric protein from stably transformed insect cells.

26. An immunogenic composition comprising a recombinant subunit influenza vaccine comprising a recombinant influenza HA-foldon protein subunit, wherein the protein subunit is expressed and secreted as a soluble protein from stably transformed insect cells.

27. An immunogenic composition comprising a recombinant subunit influenza vaccine comprising a recombinant influenza HA-foldon protein subunit, wherein the HA-foldon protein subunit is expressed and secreted as a soluble protein from stably transformed insect cells,

combined with a recombinant influenza matrix 1 protein subunit, wherein the matrix 1 protein subunit lacks a C-terminal transmembrane anchor, and is expressed and secreted as a soluble tetrameric protein from stably transformed insect cells.

28. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the influenza virus is influenza A virus.

29. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the strain of influenza virus is selected from the group consisting of H5 and H3.

30. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the carboxy-terminal portion of the hemagglutinin protein subunit is truncated within 10% of the length of a nominal ectodomain.

31. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the stably transformed insect cells are *Drosophila melanogaster* S2 cells.

32. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the immunogenic composition further comprises one or more adjuvants.

33. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the immunogenic composition further comprises one or more adjuvants selected from the group consisting of saponin and alum.

34. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the immunogenic composition further comprises GPI-0100 adjuvant.

35. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the immunogenic composition further comprises a pharmaceutically acceptable excipient.

36. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the protein subunits are purified by immuno-affmity chromatography.

37. The immunogenic composition of claim 22, 23, 24, 25, 26, or 27, wherein the immunogenic composition is administered to a subject in a vaccine.

38. The immunogenic composition of claim 22 or 24, wherein the recombinant influenza hemagglutinin ectodomain protein subunit has an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

39. The immunogenic composition of claim 22 or 24, wherein the recombinant influenza hemagglutinin ectodomain protein subunit has an amino acid sequence with at least 95% sequence identity to an amino acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

40. The immunogenic composition of claim 22 or 24, wherein the recombinant influenza hemagglutinin ectodomain protein subunit has an amino acid sequence with at least 90% sequence identity to an amino acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

41. The immunogenic composition of claim 23 or 25, wherein the recombinant influenza hemagglutinin head protein subunit has an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ DD NO:5, SEQ ID NO:6, and SEQ ID NO:7.

42. The immunogenic composition of claim 23 or 25, wherein the recombinant influenza hemagglutinin head protein subunit has an amino acid sequence with at least 95% sequence identity to an amino acid selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

43. The immunogenic composition of claim 23 or 25, wherein the recombinant influenza hemagglutinin head protein subunit has an amino acid sequence with at least 90% sequence identity to an amino acid selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

44. The immunogenic composition of claim 23 or 25, wherein the truncation points of the hemagglutinin head protein subunit are selected from the group consisting of N-terminal, C-terminal, and N-terminal and C-terminal, wherein the one or both terminal points can be varied up to 10% of the length of a nominal HA-head.

44. The immunogenic composition of claim 24, 25, or 27, wherein the recombinant influenza matrix 1 protein subunit has the amino acid sequence of SEQ ID NO: 10.

46. The immunogenic composition of claim 24, 25, or 27, wherein the recombinant influenza matrix 1 protein subunit has an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 10.

47. The immunogenic composition of claim 24, 25, or 27, wherein the recombinant influenza matrix 1 protein subunit has an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 10.

48. The immunogenic composition of claim 26 or 27, wherein the recombinant influenza HA-foldon protein subunit has an amino acid sequence selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:9.

49. The immunogenic composition of claim 26 or 27, wherein the recombinant influenza HA-foldon protein subunit has an amino acid sequence with at least 95% sequence identity to an amino acid selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:9.

50. The immunogenic composition of claim 26 or 27, wherein the recombinant influenza HA-foldon protein subunit has an amino acid sequence with at least 90% sequence identity to an amino acid selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:9.

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(71) **Applicant** *(for all designated States except US):* **CRUCELL HOLLAND B.V.** [NL/NL]; Archimedesweg 4, NL-2333 CN Leiden (NL).

(72) **Inventors; and**
(75) **Inventors/ Applicants** *(for US only):* **LUITJENS, Alfred** [NL/NL]; Archimedesweg 4-6, NL-2333 CN Leiden (NL). **VAN HERK, Herman** [NL/NL]; Archimedesweg 4-6, NL-2333 CN Leiden (NL).

(74) **Agents: VERHAGE, Richard A.** et al; P.O. Box 2048, Archimedesweg 4, NL-2333 CN Leiden (NL).

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**Declarations under Rule 4.17 :**

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(H))*

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(54) **Title:** METHOD FOR THE PRODUCTION OF AD26 ADENOVIRAL VECTORS

(57) **Abstract:** The invention provides methods for large-scale production of recombinant adenovirus 26, using perfusion systems and infection at very high cell densities.

Title: Method for the production of Ad26 adenoviral vectors

The invention relates to the field of cell culture and adenovirus production. More particularly, it concerns improved methods for the culturing of mammalian cells, infection of those cells with adenovirus and the production of adenovirus particles therefrom.

## Background of the invention

Recent developments in the field of DNA vaccination using recombinant viral vectors have created the need for large scale manufacturing of clinical grade material. Processes are needed to be able to support the less and least developed world with sufficient amounts of recombinant adeno-based vaccines to fight e.g. the Tuberculosis and Malaria problem in the world. An evaluation of the birth cohort shows that more than 150.000.000 births are expected for the less and least developed world in 2010-2015. Based on this birth cohort the projected annual demand for a vaccine could reach approximately $1.5 \times 10^{19}$ virus particles (VP) on a yearly basis (http://esa.un.org/unpp/index. asp?panel=2).

Several processes for production of adenoviruses have been described. These processes use adherent cell cultures in roller bottles, cell factories (Nunclon from Nunc or CellStack from Corning), or Cell Cubes (Corning). Production processes on adherent cell cultures cannot fulfill the worldwide demand for adeno-based vaccines. Therefore the cells used in the adherent process are adapted to suspension cultures (e.g. HEK293 and PER.C6® cell lines). With the use of suspension cultures it is possible to scale-up production processes to large-scale bioreactors. Suspension cell cultures for adenovirus production are routinely achieved between 3 to 20L scale and successful scale-up has been reported up to 100L (Kamen et al, 2004), and 250L (Xie et al, 2003). Experiments are reported in which scaling up to 10.000L is anticipated (Xie et al, 2003).

However, a major disadvantage of scaling up to 10.000L is the high capital investment (CAPEX), which is needed to design and build a 10.000L bioreactor facility. Furthermore, the CAPEX commitment of building a 10.000L facility, under BSL 2 conditions, must be realized before even knowing if the product will be successful (Phase IV and beyond). The total investment cost for a 10.000L bioreactor

plant is reported between C225.000.000 and C320.000.000 (Estape et al, 2006). Therefore, preparation at lower scale, e.g. in 1000L or smaller bioreactors, would be desirable.

With the use of currently existing processes, more than 150 batches at 1000L scale a year must be produced in order to reach the target of $1.5xl0^{19}$VP/year. Therefore, a need exists to improve systems for adenovirus production, to improve yields of adenovirus particles in order to fulfil the world-wide demand of adenovirus vaccines, preferably at non-prohibitive costs.

One of the issues encountered in adenovirus production optimization is the so-called "cell density effect". In batch-mode operation, several references suggest the existence of an optimal cell density at infection for adenovirus production. The optimum lies between 0.5 - $lxlO^6$ cells/mL (Maranga et al, 2005; Kamen et al., 2004). It was shown for adenovirus (Ad5) production in a batch stirred tank bioreactor that the virus productivity per cell remains constant up to around $0.9xl0^6$ cells/mL, but drops abruptly at around $lxlO^6$ cells/mL (Altaras et al, 2005). Beyond $2xl0^6$ cells/mL, no infectious particles were detectable. The breakpoint related to specific production drop with cell densities at infection is medium dependent. No available commercial medium to date has shown potential to support high yields of virus particles, while maintaining the specific production optimal at cell densities beyond $lxlO^6$ cells/mL (Kamen et al, 2004). The reasons for this drop is not known yet but might be due to limited nutrient availability for virus production, or due to high metabolites concentrations that are inhibitory for virus production.

Fed-Batch operations, like addition of glucose, glutamine and amino acids allowed infections at cell densities up to $2xl0^6$ cells/mL. However, the productivities attained at high cell densities were lower than those obtained with infection at cell densities of $lxlO^6$ cells/mL (Kamen et al., 2004).

In perfusion processes the cells are retained in the bioreactor by hollow fibers, spin filters or acoustic separators while culture medium is perfused through the bioreactor. In these processes cell densities of $>100xl0^6$ cells/mL can sometimes be reached (e.g., Yallop et al, 2005).

Infected perfusion cells showed premature cell loss during perfusion with a hollow fiber system. This might be related to their higher shear sensitivity due to the viral infection (Cortin et al., 2004). The hydro-dynamical stresses induced in the tubing, the hollow fibers, or the peristaltic pump on more fragile, infected cells was

most likely the cause for this phenomenon. Since infected cells are more fragile, particularly the acoustic separator (Henry et al, 2004) has been suggested to be desirable if the perfusion is to be maintained throughout the infection phase. However, infections performed in perfusion mode could only be maintained for cell densities up to $3xl0^6$ cells/mL with a perfusion rate of 2 vol/day. Infection at a cell density of $6x10^6$ cells/mL led to a fivefold reduction in specific productivity (Henry et al, 2004).

Despite the reported cell density effect by others, one report (Yuk et al, 2004) described successful perfusion cultures of human tumor cells as a production platform for oncolytic adenoviral vectors. That report described a high-cell-density perfusion process using alternating tangential flow (ATF) technology. At an average viable cell density at infection of $9 \times 10^6$ HeLaS3 cells/mL, an average viral titer of about $4 \times 10^{11}$ VP/mL was observed. The tumor cells used in that report are not preferred as production cells, since use of tumor cells may pose safety risks when the produced adenovirus particles are to be administered to humans. The recombinant adenovirus in that report was based on Ad5. Such adenoviruses have limited possibilities for use as vaccines since a majority of the human population contain pre-existing neutralizing antibodies against Ad5, and recombinant adenoviruses from other serotypes are therefore more suitable for use as vaccines (see e.g. WO 00/70071). Recombinant adenoviruses especially advantageous for use as vaccines include Ad26 (WO 00/70071).

Limited information, if any, is available for the large scale production of recombinant adenoviruses from other serotypes than Ad5, in particular for the advantageous serotype 26. Some differences between Ad35 and Ad5 production at large scale have been described previously in e.g. PCT/EP2009/064265. The somewhat different physical properties of recombinant adenoviruses of different serotypes may give rise to differences in production processes. Such potential differences may especially be important at industrial scale, where even seemingly small differences at small scale may have large economic consequences on the scale envisaged for production of the annual world-wide demand. For instance, it was shown by the applicant that the reported cell density effect for Ad5 was different for Ad35 (PCT/EP2009/064265). Thus, rAd35 propagates differently in producer cells than rAd5 during large scale production processes. Apparently, the propagation of adenoviruses from different serotypes is very unpredictable.

In order to fulfil the world-wide demand of recombinant adenovirus serotype 26 (rAd26) vaccines, a need exists to improve systems for rAd26 production. The present invention provides improved methods for industrial production of rAd26.

## Summary of the invention

We have found herein that yet another serotype, i.e. Ad26 behaves differently than other serotypes Ad5 and Ad35. Indeed, Ad26 tends to show a slight cell density effect, yet not as accentuated as the density effect seen for Ad5. In addition, cells that are infected with Ad26 tend to grow further after infection, while cells infected with Ad35 show a decreased growth post infection.

These results again suggest that processes for specific adenovirus serotypes may have to be fine-tuned for each serotype, in order to obtain optimal results. The present invention provides an optimized system for production of rAd26 in terms of yield, quality of the rAd26 obtained, and ease of handling of the harvest for down stream processing.

The invention provides a method for producing recombinant adenovirus serotype 26 (rAd26), the method comprising: a) culturing producer cells in suspension with a perfusion system; b) infecting said cells at a density of between about $10x10^6$ viable cells/mL and $16x10^6$ viable cells/mL with rAd26; c) further culturing the infected cells with a perfusion system to propagate said rAd26; and d) harvesting said rAd26.

In certain embodiments said cells in step b) are infected with rAd26 at a density of between about $10x10^6$ and $14x10^6$ viable cells/mL.

In certain preferred embodiments, said perfusion system in step c) is an alternating tangential flow (ATF) perfusion system. In other preferred embodiments, said perfusion system in step a) is an alternating tangential flow (ATF) perfusion system. In a preferred embodiment, said perfusion system in both steps a) and c) is an alternating tangential flow (ATF) perfusion system.

In certain embodiments, the method of the invention further comprises: e) purifying the rAd26. In further embodiments, the method further comprises: f) preparing a pharmaceutical composition containing the purified rAd26.

In certain embodiments, said recombinant adenovirus lacks at least a portion of the E1 region, and comprises heterologous nucleic acid.

In preferred embodiments, the physical particle to infectious particle (VP/IU) ratio of the produced rAd26 is less than 30:1, preferably less than 20:1.

It is also an aspect of the invention to provide a method for producing at least lxl0 $^{12}$ rAd26 virus particles (VP)/mL, the method comprising: a) culturing producer cells in suspension with a perfusion system; b) infecting said cells at a density of between about lOxlO$^{6}$ viable cells/mL and 16xl0$^{6}$ viable cells/mL with rAd26; c) further culturing the infected cells with a perfusion system to propagate said rAd26, whereby the concentration of rAd26 virus particles reaches at least lxlO $^{12}$ VP/mL; and d) harvesting said rAd26.

The invention also provides a bioreactor with a working volume of between 2L and 1000L, comprising: culture medium, producer cells, and at least lxl 0$^{12}$ rAd26 virus particles (VP)/mL. In certain embodiments, the bioreactor has a working volume of between 50L and 500L. In preferred embodiments, the bioreactor is connected to an ATF perfusion system.

## Brief description of the Figures

FIG. 1. Infection at high cell density in shakers with rAd5.

FIG. 2. Infection at high cell density in shakers and 2L bioreactor with rAd35.TB-S.

FIG. 3. Infection at high cell density in shakers with rAd26.

FIG. 4. Cell growth post infection with rAd26.

FIG. 5. Cell growth post infection with rAd35.

## Detailed description of the invention

The present invention describes a new process for the production of large quantities of recombinant adenovirus rAd26. This optimized process relies on the ability to infect cultures at high cell density with preservation of a high virus productivity per cell. Herewith, it offers a method to obtain a harvested virus solution with high virus concentration in a single bioreactor. Typical yields of current processes for rAd26 are about 2-3x10$^{11}$ VP/mL. Indeed, it is believed that very large quantities of rAd26 particles can be produced using the processes of the present invention, for instance quantities of at least about 5x10$^{11}$ VP/mL, preferably at least about 6, 7, 8, or 9xlO$^{11}$ VP/mL. Preferably at least lxlO $^{12}$ VP/mL of rAd26 are produced, more preferably at least 1.5x10$^{12}$ VP/mL, still more preferably at least 2xl0 $^{12}$ VP/mL, e.g. between about lxlO $^{12}$ and 5xl0 $^{12}$ VP/mL. Typically, the process

**Claims**

1. A method for producing recombinant adenovirus serotype 26 (rAd26), the method comprising:

a) culturing producer cells in suspension with a perfusion system;

b) infecting said cells at a density of between $1Ox1O^6$ viable cells/mL and $16x10^6$ viable cells/mL with rAd26;

c) further culturing the infected cells with a perfusion system to propagate said rAd26; and

d) harvesting said rAd26.

2. A method according to claim 1, wherein said cells in step b) are infected with rAd26 at a density of between about $1OxlO^6$ and $14x10^6$ viable cells/mL.

3. A method according to any one of the preceding claims, wherein said perfusion system in step c) is an alternating tangential flow (ATF) perfusion system.

4. A method according to any one of the preceding claims, further comprising:

e) purifying the rAd26, and optionally

f) preparing a pharmaceutical composition containing the purified rAd26.

5. A method according to any one of the preceding claims, wherein said recombinant adenovirus lacks at least a portion of the E1 region, and comprises heterologous nucleic acid.

6. A method according to any one of the preceding claims, wherein said perfusion system in step a) is an alternating tangential flow (ATF) perfusion system.

7. A method according to any one of the preceding claims, wherein step a) is performed in a first bioreactor, and steps b) and c) are performed in a second bioreactor.

8. A method according to any one of the preceding claims, wherein the physical particle to infectious particle (VP/IU) ratio of the produced rAd35 is less than 30:1, preferably less than 20: 1.

9. A bioreactor comprising culture medium, producer cells, and virus particles wherein said bioreactor has a working volume of between 2L and 1000L, preferably between 50L and 500L, and characterized in that said bioreactor comprises at least lxlO$^{12}$ rAd26 virus particles (VP)/mL.

10. A bioreactor according to claim 9, connected to an ATF perfusion system.

11. A bioreactor according to claim 9 or 10, wherein the rAd26 virus particles have a VP/IU ratio of less than 30:1, preferably less than 20: 1.

12. A method for producing at least lxlO$^{12}$ rAd26 virus particles (VP)/mL, the method comprising:

a) culturing producer cells in suspension with a perfusion system;

b) infecting said cells at a density of between lOxlO$^6$ viable cells/mL and 16xl0$^6$ viable cells/mL with rAd26;

c) further culturing the infected cells with a perfusion system to propagate said rAd26, whereby the concentration of rAd26 virus particles reaches at least lxlO$^{12}$ VP/mL; and

d) harvesting said rAd26.