

CLARIFICATION OF CELL CULTURE BROTH USING SINGLE USE DISPOSABLE BIOSETTLERS

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Key Words: Clarification of Cell Culture Broth, Single Use Disposable BioSettlers, Compact Inclined Settlers, Selective Cell Retention Device, Continuous Perfusion Bioreactors.

Enhanced sedimentation on inclined surfaces has been exploited successfully in mammalian cell cultures to recycle live and productive mammalian cells into the continuous perfusion bioreactors, while removing selectively the smaller dead cells and cell debris into the harvest stream. We have developed a compact settler design using cylindrical and conical geometries, with about 5-10X more settling surfaces over a given footprint, compared to the traditional rectilinear scale up design. With this improved design, we have demonstrated that these compact settlers can also recycle smaller microbial yeast cells back to the perfusion bioreactors operated continuously over two months. We have fabricated the compact settler as a single use disposable plastic settler at an initial size of 150 mm diameter (“BioSettler150”). Perfusion bioreactors attached with this BioSettler as the selective cell retention device achieve high cell densities and viabilities of CHO cells over extended culture durations by selectively removing smaller dead cells and cell debris.

We have now successfully adapted our BioSettler to clarify cell culture broth from end of fed-batch culture, recovering over 95% of secreted antibody product in the clarified harvest stream and reducing the turbidity of cell culture broth by ~ 80% in clarified harvest stream. This primary clarification using single use disposable BioSettler is a very gentle process, without any measurable increase in cell lysis (or LDH activity). Our clarification process is accelerated by faster settling of cell clumps, formed due to acidification of cell culture broth to a pH value of 5 or less, which can be carried out within the bioreactor at the end of fed-batch culture or in the BioSettler. If the pH is lowered to 3.5 for the requisite period of time within the bioreactor or BioSettler, our cell aggregation and clarification process can replace a later low pH viral inactivation step, moving it closer to where any adventitious viral replication may occur. The BioSettler can be scaled up to clarify the cell culture broth from 2 - 5kL single use bioreactor within 4-6 hours.



Antibody recovery and turbidity reduction results for clarification with BioSettler150 are shown below:

Sample Description	PCV corrected Volume(ml)	IgG (measured on Roche CEDEX Bioanalyzer)			Total Protein (measured using BCA)			PCV (%)	Turbidity (NTU)	Flow rate (L/hr)	
		Conc. (mg/l)	Total(mg)	% of Start	Conc. (mg/l)	Total(mg)	% of Start				
CultureBroth	8716	106	923.9	100	2275.8	19836.7	100	5	528		
Fraction 1	998	100	99.8	10.8	1998.9	1994.8	10.1	0.21	117.2	2.2	
Fraction 2	998	98	97.8	10.6	1977.1	1973.4	9.9	0.19	101.6	2.6	
Fraction 3	998	103	102.8	11.1	1972.4	1969.1	9.9	0.17	96.3	3	
Fraction 4	1098	103	113.1	12.2	1998.3	2194.5	11.1	0.17	93.9	3.3	
Fraction 5	998	99	98.8	10.7	2036.1	2032.6	10.2	0.17	111.5	3.5	
Fraction 6	998	99	98.8	10.7	2128	2124.5	10.7	0.17	110.4	3.5	
Final Aspirate	2794	105	293.3	31.7	2227.5	6222.9	31.4	0.23	106.8		
% Recovery		97.9			93.3						