

6-12-2022

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AFFINITY PURIFICATION OF SARS-COV-2 SPIKE PROTEIN RECEPTOR BINDING DOMAIN PRODUCED IN A C1 FUNGAL EXPRESSION SYSTEM

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Key Words: downstream processing, affinity chromatography, purification of recombinant proteins

The Receptor Binding Domain (RBD) of the spike protein of SARS-CoV-2 has shown promise for diagnosis, treatment, and development of vaccines for COVID-19. However, two problems persist with large scale production of RBD: 1) lack of high productivity upstream cell culture, 2) absence of a commercial, highly selective affinity resin. In an effort to overcome these limitations, we evaluated two novel technologies for the production and purification of RBD.

Briefly, RBD was expressed using C1, an engineered fungal strain of *Thermothelomyces heterothallica* (DyadicInternational¹). The C1 platform expresses glycosylated antigens with high productivity, stability, and purity. RBD was purified using a novel affinity resin² known to produce yields of 90% to 95% purity in one chromatography step. Affinity purification did not affect protein quality, as demonstrated by ACE-2 binding of RBD. The novel affinity resin showed excellent base stability, consistent product quality, and similar ACE-2 binding activity over 40 cycles.

RBD produced in C1, in conjunction with affinity purification using a novel affinity resin, provides a breakthrough in the large-scale production of affordable COVID-19 protein-based vaccines.

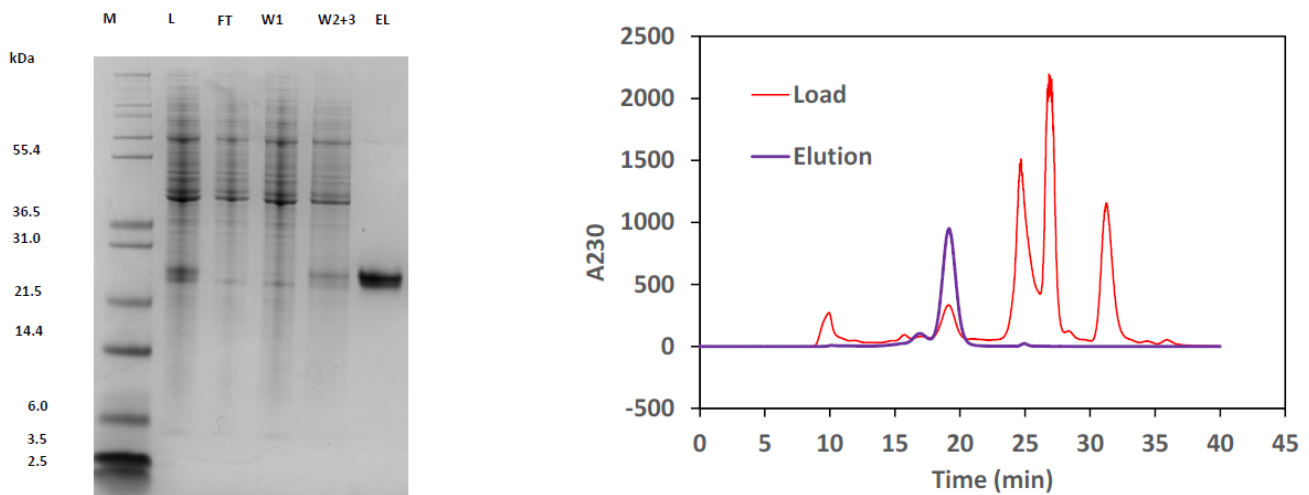


Figure 1 – A column packed with 1 mL NGL COVID-19 Spike Protein Affinity Resin was used for purification of C1 generated RBD. The load (L), flow through (FT), wash (W1, W2+3), and elution (EL) streams were analyzed using SDS Page (left) and size exclusion chromatography (SEC) (right).

¹ C1 is an engineered *Thermothelomyces heterothallica* fungus developed for high productivity, increase stability and purity by Dyadic International, Inc., 140 Intracoastal Pointe Drive, Jupiter, Florida 33477 USA.
² The novel affinity resin for the purification of the SARS-CoV-2 Spike RBD Protein for vaccine development and manufacturing was co-developed by Repligen Corporation and Navigo Proteins GmbH.