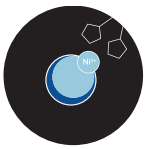
Purification of SARS-CoV-2 Spike RBD Using Nuvia IMAC Resin



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In the midst of the COVID-19 pandemic, vaccinations and antibody treatments became urgently necessary to tackle SARS-CoV-2, and the receptor binding domain (RBD) of the viral spike protein was found to be a significant target of neutralizing antibodies. For applications in vaccines and diagnostics, researchers have discovered techniques to produce the spike RBD in insect cells in high yields. In 2022, Poodts et al. published the study *Improved Expression of SARS-CoV-2 Spike RBD Using the Insect Cell-Baculovirus System*, in which the researchers describe using a baculovirus insect cell culture to enhance the synthesis of a polyhistidine (His6)-tagged SARS-CoV-2 RBD. The researchers purified Sf9 cell lysate containing recombinant His6-tagged RBD (rRBD) using immobilized metal affinity chromatography (IMAC) purification with Nuvia IMAC Resin (Bio-Rad Laboratories, Inc.). The researchers had ample RBD yields to use for further studies. Here, we demonstrate the effective capture and purification of the His6-tagged RBD using Nuvia IMAC Resin in accordance with the procedures outlined by Poodts et al.

Methods

A recombinant baculovirus with the SARS-CoV-2 spike RBD and a C-terminal His6 tag was used to infect Sf9 insect cells (Thermo Fisher Scientific Inc.), while the virus was expressed using the Bac-to-Bac Baculovirus Expression System (Thermo Fisher Scientific). The cell culture supernatant containing secreted RBD was recovered 96 hr after infection and diafiltered with equilibration buffer (20 mM phosphate buffer, pH 8.0, 300 mM NaCl, 20 mM imidazole). The supernatant was loaded onto a Nuvia IMAC Resin–packed column using an AKTA pure Chromatography System (Cytiva). After loading, the column was washed with 80 mM imidazole and eluted by increasing the imidazole concentration to 500 mM. Fractions were analyzed by SDS-PAGE and western blot using an anti-S antibody serum, and a mouse anti-equine immunoglobulin conjugated with HRP (Sigma-Aldrich) as the secondary antibody under reducing conditions.

Results

Nuvia IMAC Resin demonstrated a higher binding capacity for the His6-tagged SARS-CoV-2 spike RBD than the published dynamic binding capacity of the resin of over 40 mg/ml for His6-tagged proteins. The purification fractions' Coomassie-stained SDS-PAGE analysis is shown in Figure 1. The elution fractions had an enhanced target of 30 kDa RBD. Based on the findings of Poodts et al., a single chromatographic step is predicted to yield >95% purity. In this study, 21.1 ± 3.7 mg/L of purified rRBD culture was obtained with a yield of 82%, which is the highest yield reported to date for Sf9 and High Five Cells (Thermo Fisher Scientific Inc.) infected with recombinant baculovirus.

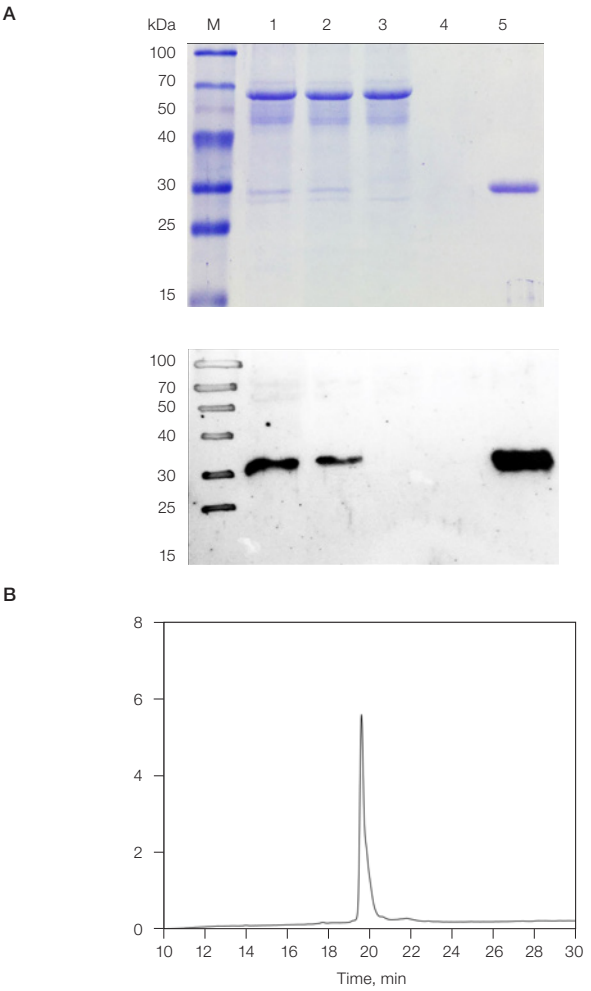

Fig. 1. Analysis of fractions collected during the Nuvia IMAC Resin purification process of His6-tagged receptor binding domain (rRBD). A, SDS-PAGE and western blot analysis of anti-S antibody fractions under reducing conditions. M, protein marker; 1, Sf9 cell expression supernatant; 2, diafiltered sample; 3, flow-through; 4, washing step; 5, IMAC elution fraction. **B**, RP-HPLC analysis of rRBD purified by Nuvia IMAC Resin.

Key Findings

High Yield and Purity — In the purification of SARS-CoV-2 spike RBD, Nuvia IMAC Resin performed remarkably well. The target protein was successfully collected by the chromatography column, producing high yields with little contamination from host cell proteins and other contaminants.

Optimized Conditions — To obtain the desired purity and yield, researchers can readily optimize the binding and elution conditions. This adaptability is necessary to modify the procedure to meet certain research or industrial needs.

Batch Consistency — Nuvia IMAC Resin demonstrated repeatability and dependability in the generation of SARS-CoV-2 spike RBD by consistently providing high-quality purification across several purification runs.

Conclusions

The SARS-CoV-2 spike RBD, a crucial vaccine and treatment target, can be expressed in a robust manner thanks to the insect cell–baculovirus system. As demonstrated here, Nuvia IMAC Resin offers excellent purification, making it possible to effectively capture and isolate the His6-tagged RBD protein. Nuvia IMAC Resin is a reliable option for purification from this and other recombinant insect cell–baculovirus systems.

Reference

Poodts J et al. (2022). Improved Expression of SARS-CoV-2 Spike RBD Using the Insect Cell-Baculovirus System. Viruses 14, 2794.

Acknowledgment

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Visit bio-rad.com/NuviaIMAC for more information and to request samples.

Note: This article is based on the study conducted by Poodts et al. (2022) and is intended for informational purposes only. It is recommended to consult the original study and perform additional validation experiments according to specific requirements.

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