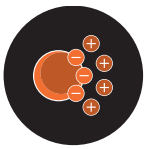
Purification of SARS-CoV-2 Spike Ectodomain Protein Using High-Throughput Screening and Non-Affinity Methods with Nuvia HR-S Chromatography Resin



CATION EXCHANGE

The purification of recombinant proteins is a crucial step in various biotechnological applications, including the production of diagnostic, therapeutic, and vaccine targets, as well as the resulting vaccines and biopharmaceutical therapies themselves. The COVID-19 pandemic has highlighted the urgent need for efficient and scalable purification methods for viral proteins. In 2022, Cibelli et al. from the National Institutes of Health published *Advances in purification of SARS-CoV-2 spike ectodomain protein using high-throughput screening and non-affinity methods*, highlighting the successful use of Nuvia HR-S Resin for the purification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike ectodomain protein. Presented here is a summary of their methods and findings, demonstrating that this high-resolution (HR) cation exchange resin is well-suited for many biopharma workflows.

Method Development

The authors recombinantly expressed a construct of the SARS-CoV-2 spike (S) ectodomain protein (the target protein) in a Chinese hamster ovary (CHO) host cell system. After cell culture, the supernatant containing the target protein was collected, clarified, filtered, and subjected to an initial non-affinity capture chromatography method. The initial chromatography step removed a majority of the impurities and concentrated the target protein. However, the authors deemed additional purification necessary for their desired application. Figure 1 shows an overview of the workflow.

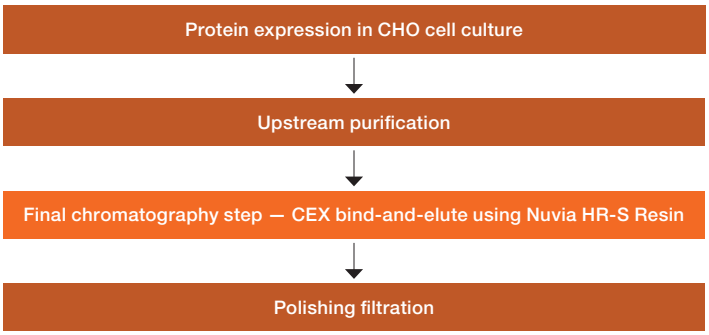


Fig. 1. Workflow overview of SARS-CoV-2 spike ectodomain protein purification using Nuvia HR-S Resin as the second chromatography step. CEX, cation exchange; CHO, Chinese hamster ovary.

Cibelli et al. developed a high-throughput screening (HTS) platform to identify chromatographic resins suitable for further polishing of the target SARS-CoV-2 spike ectodomain protein. The authors evaluated various resins based on their dynamic binding capacity (DBC), purification yield, and the resulting purity of the protein. Among the resins tested, Nuvia HR-S Resin demonstrated promising results, providing high DBC and excellent purification performance. As shown in Figure 2, the protein eluted from Nuvia HR-S Resin from 100 to 200 mM NaCl, and showed high purity using the Pico Protein Express Assay and the LabChip GXII Touch Capillary Electrophoresis System, indicating utility as a bind-and-elute polishing step.

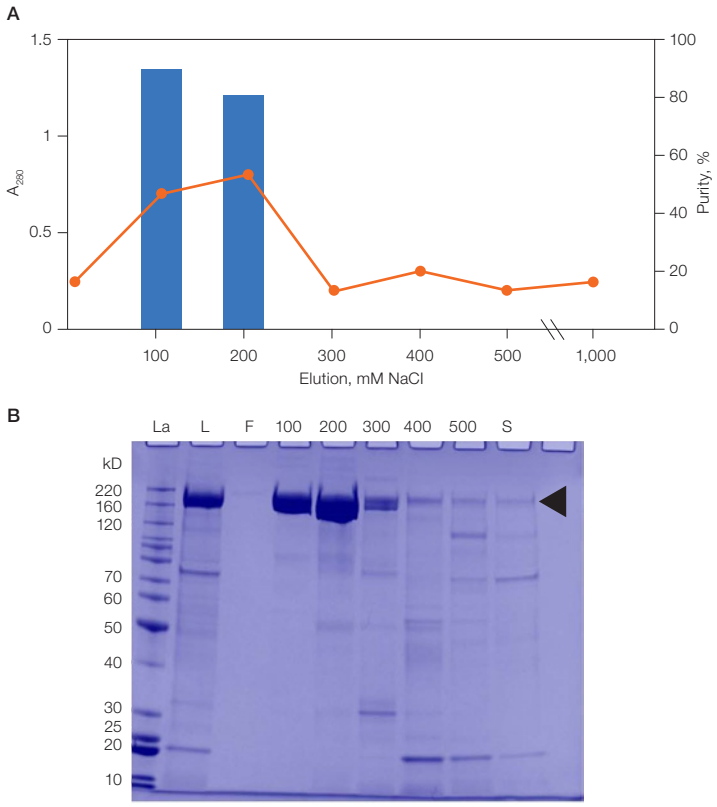


Fig. 2. Cation exchange high-throughput screen and proof of concept. **A**, HTS bind-and-elute chromatography of SARS-CoV-2 spike ectodomain protein using Nuvia HR-S Resin. A_{280} (—) and LabChip Pico Protein Express Assay (Revvity) results (■) plotted against elution NaCl concentration. **B**, SDS-PAGE evaluation of step elution fractions from Nuvia HR-S Resin using 100 to 500 mM NaCl. SARS-CoV-2 spike ectodomain protein (◀). La, standards ladder; L, load; F, flowthrough; S, strip.

A Two-Step Process Workflow Using Nuvia HR-S Chromatography Resin

A chromatography column packed with Nuvia HR-S Resin was equilibrated with the appropriate buffers. The column dimensions and bed height were chosen based on the experimental setup of the study. The concentrated protein sample obtained from the initial capture step was applied to the Nuvia HR-S column after being equilibrated with 37.5 mM sodium citrate and 50 mM NaCl, pH 4.0. The loading volume and flow rate were optimized for maximum binding efficiency and to minimize processing time. After sample loading, the column was washed to remove nonspecifically bound impurities. The composition and volume of the wash buffer were set to achieve optimal purity. Based on the tests, the resin Toyopearl SP-650 Resin (Tosoh Bioscience LLC) was selected for use in flow-through mode with 50 mM sodium citrate and 50 mM NaCl, pH 4.0. The Toyopearl SP-650M Resin flow-through material was immediately loaded onto the Nuvia HR-S column and eluted with 50 mM sodium citrate and 180 mM NaCl, pH 4.0.

The SARS-CoV-2 spike ectodomain protein was eluted from the Nuvia HR-S column using an elution buffer of 50 mM sodium citrate, pH 4.0, with steps from 180 to 250 mM NaCl. Under these conditions, Nuvia HR-S elution buffer conductivity investigations showed a recovery rate of >80%, with host cell protein (HCP) levels rising with increasing NaCl concentrations (Figure 3). To reduce relative HCPs and keep recovery levels high, the final Nuvia HR-S elution buffer contained 180 mM NaCl. The elution conditions were selected to achieve a high target protein yield while maintaining its structural integrity and purity. Elution fractions underwent SDS-PAGE analysis, protein quantitation using a binding assay on the Octet BLI Label-Free Detection System (Sartorius AG), and enzyme-linked immunosorbent assays (ELISA) for HCP clearance.

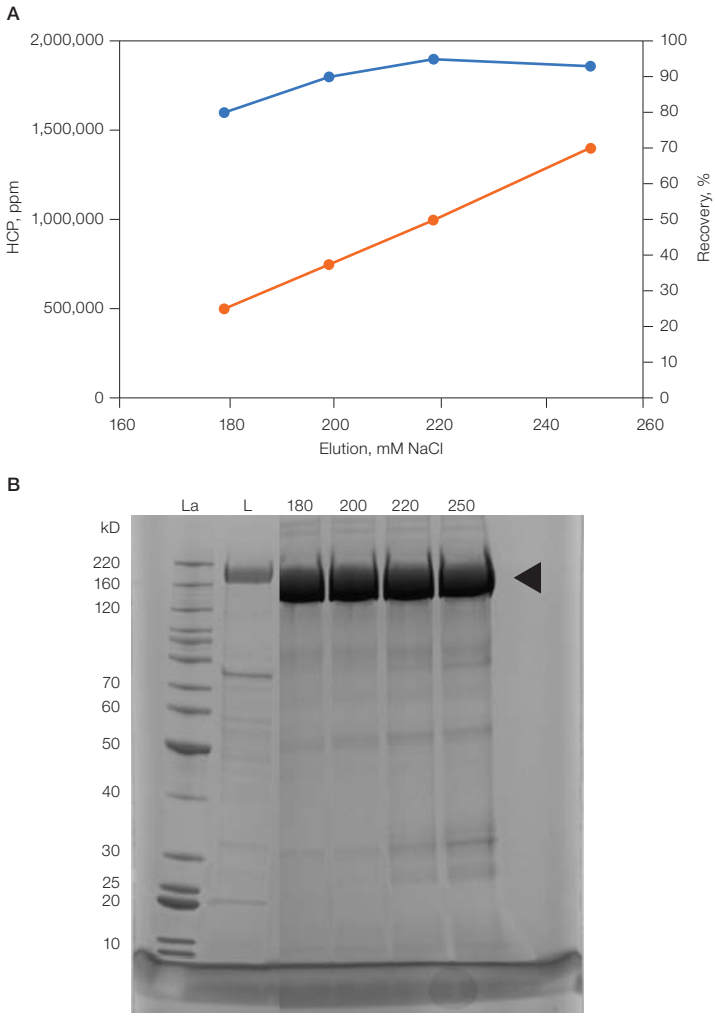


Fig. 3. Nuvia HR-S elution buffer selection. A, HCP (—), and % recovery (—) are plotted against the stepped elution with NaCl ranging from 180 to 250 mM NaCl. **B**, samples eluted from 180 to 250 mM NaCl assessed by SDS-PAGE. SARS-CoV-2 spike ectodomain protein (◀). La, standards ladder; L, load.

Analytics Used

During the purification process, various analytics were employed to assess the quality and purity of the SARS-CoV-2 spike ectodomain protein. UV spectroscopy was used to monitor protein elution peaks, calculate protein concentration, and assess the presence of impurities. SDS-PAGE was utilized to evaluate protein integrity, molecular weight, and to detect any remaining impurities. Size exclusion chromatography (SEC) coupled with high performance liquid chromatography (HPLC) was employed to analyze the

monomeric form and aggregation state of the purified SARS-CoV-2 spike ectodomain protein. Recovery was calculated using a biolayer interferometry platform (Octet BLI Label-Free Detection System). These analytics provided crucial information regarding the purity, yield, structural integrity, and overall quality of the purified SARS-CoV-2 spike ectodomain protein.

Results

Nuvia HR-S Resin is a strong cation exchange resin designed for high-resolution separations and high-throughput applications. It exhibits excellent binding capacity for proteins, including the SARS-CoV-2 spike ectodomain protein. In the study by Cibelli et al., Nuvia HR-S Resin demonstrated superior performance compared to other tested resins, allowing for efficient purification of the target protein with high yield and purity.

Key Findings

High dynamic binding capacity — Nuvia HR-S Resin exhibited a high DBC for the SARS-CoV-2 spike ectodomain protein, enabling the purification of large quantities of the target protein per resin volume, thus improving process productivity.

High purity — Nuvia HR-S Resin demonstrated excellent selectivity, effectively removing impurities and achieving high purity of the SARS-CoV-2 spike ectodomain protein. This is critical for downstream applications, ensuring the quality and efficacy of the purified protein.

Scalability — Nuvia HR-S Resin is available in a range of formats suitable for both laboratory-scale and large-scale applications. This enables seamless process transfer and scalability, facilitating the purification of the SARS-CoV-2 spike ectodomain protein.

Conclusions

Cibelli et al. demonstrated the efficacy of Nuvia HR-S Chromatography Resin for the purification of the SARS-CoV-2 spike ectodomain protein. Nuvia HR-S Resin exhibited high binding capacity, excellent purity, and scalability, making it an ideal choice for the purification of viral proteins. The utilization of Nuvia HR-S Resin can enhance the efficiency and productivity of protein purification workflows, supporting the development of vaccines, therapeutics, and diagnostic tools for combating the COVID-19 pandemic.

Reference

Cibelli N et al. (2022). Advances in purification of SARS-CoV-2 spike ectodomain protein using high-throughput screening and non-affinity methods. Sci Rep 12, 4458.

Visit **bio-rad.com/NuviaHRS** for more information on Nuvia HR-S Chromatography Resin and its applications, or contact our technical support team.

Note: This article is based on the study conducted by Cibelli 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.

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23*
France *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 080 007 7373* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

Bulletin 3583 Ver B US/EG *24-0368 0524 Sig 0124*