CHROMATOGRAPHY

Nuvia aPrime 4A Hydrophobic Anion Exchange Resin

- Wide design space
- Optimal product recovery
- Mechanical and chemical stability
- Full regulatory support

High Purity and Recovery in Downstream Purification Processes

Nuvia aPrime 4A Hydrophobic Anion Exchange Resin possesses the optimal balance of ion exchange and hydrophobic interactions to deliver simultaneous purity and yield of therapeutic proteins and monoclonal antibodies that are difficult to purify.

Benefits of Nuvia aPrime 4A Resin

- Removes multiple impurities, such as viruses, host cell proteins, and aggregates and nucleic acids in a single chromatography step
- Enables capture and polish purification without extensive feedstream conditioning, saving process time and cost
- Facilitates straightforward method development

Nuvia aPrime 4A is designed with a positively charged hydrophobic ligand (Figure 1). It can operate across a wide range of salt concentrations and pH. The resin's ligand density and hydrophobicity are designed to facilitate selective and reversible binding of target molecules for maximal purity and recovery. The technical properties of Nuvia aPrime 4A Resin are listed in Table 1.

Nuvia aPrime 4A can be used to purify both established therapeutic proteins and diverse new constructs in development, including those which lack affinity handles. It is also effective in the purification of salt- and pH-sensitive proteins with a high propensity for aggregation and/or degradation.

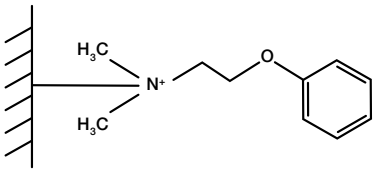

Fig. 1. Mixed-mode ligand for Nuvia aPrime 4A Resin.

Table 1. Properties of Nuvia aPrime 4A Resin.

Property	Description
Functional group	Aromatic hydrophobic anion exchanger
Base matrix composition	Macroporous highly crosslinked polymer
Median particle size	50 ± 10 µm
Ligand density	100 ± 20 µeq/ml
Dynamic binding capacity (DBC)*	≥50 mg/ml at 300 cm/hr
Recommended linear flow rate	50–300 cm/hr
Pressure-flow performance**	Under 3 bar at flow rate of 300 cm/hr
Compression factor	1.1–1.25
pH stability***	Short-term: 2–14
Shipping solution	20% ethanol + 1 M NaCl
Regeneration	1 M NaOH
Sanitization	1 M NaOH
Storage conditions	20% ethanol
Storage temperature	Room temperature
Chemical stability†	1 M NaOH, 1 M HCl, 25% acetic acid, 8 M urea, 6 M guanidine HCl, 3 M NaCl, 1% Triton X-100, 20–70% ethanol, 30% isopropanol
Shelf life††	5 years

* 10% breakthrough capacity determined with 1.0 mg/ml of an acidic monoclonal antibody (mAb; pI ~6.9) in 20 mM $NaPO_4$, pH 7.8.
** 20 x 20 cm packed bed (1.25 compression factor).
*** No significant change in ligand density after 200 hr contact at 22ºC.
† No significant change in ligand density after 1 week contact at 22ºC.
†† Stored at room temperature in 20% ethanol + 1 M NaCl.

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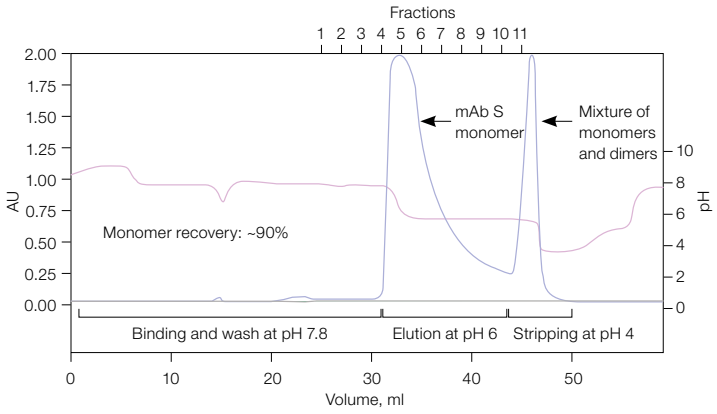
Exceptional Purity and Recovery

The Nuvia aPrime 4A bead is built to provide a good DBC and excellent recovery of biomolecules under mild conditions. The 50 µm particle size offers good resolution and high productivity while keeping the column pressure relatively low.

The ligand density and hydrophobicity of Nuvia aPrime 4A have been fine-tuned to facilitate selective and reversible binding of target molecules in order to achieve maximal purity and recovery. We tested the ability of this resin to clear high molecular weight species from an acidic mAb (pI ~6.9) feedstream. At pH 7.8, Nuvia aPrime 4A interacts with the mAb through both electrostatic and hydrophobic interactions. The mAb monomer was eluted at ~pH 6 with an overall recovery of ~90%. The high molecular weight impurities were retained until the resin was stripped with a pH 4 buffer (Figure 2).



Sample	Monomer, %	Dimer, %	Tetramer, %
Load	93.1	6.5	0.4
Eluate	100	ND	ND
Strip	76.8	23.2	ND

Fig. 2. Purification of mAb S (pI ~6.9) on Nuvia aPrime 4A Resin. A solution containing ~10 mg of target mAb in 20 mM $NaPO_4$, pH 7.8 was injected onto a 1 ml column and eluted by a buffer at pH 6. Clear separation of the high molecular weight impurities from the monomeric fraction was observed. AU (—); pH (—). AU, absorbance unit; ND, not done.

Straightforward Method Development

The mixed-mode nature of the Nuvia aPrime 4A ligand facilitates straightforward method development and process optimization. A simple design of experiment protocol can also be used by method developers to optimize their process conditions. We tested the ease of method development for purification of a basic protein with a pI of ~8.45. Our goal was to find the optimal conditions for the highest monomer recovery and purity. A fractional factorial screening design with two center points was performed. Binding capacity was low under all tested conditions (Figure 3A), suggesting that flow-through purification was more appropriate for this protein. Figures 3B and 3C show the high molecular weight impurity clearance and monomer recovery in response to purification buffer composition (or purification condition).

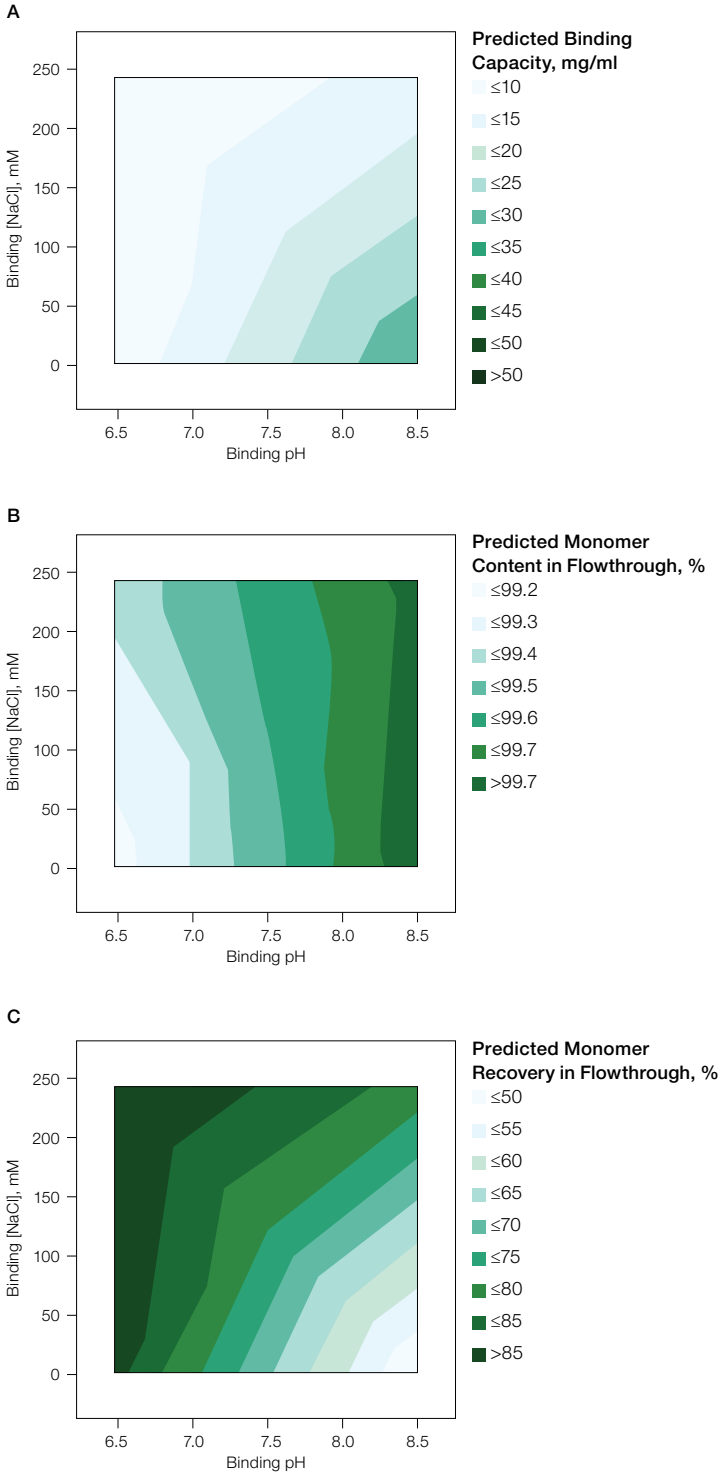


Fig. 3. Basic protein (pI ~8.45) purification using Nuvia aPrime 4A Resin. **A**, effect of binding buffer pH and NaCl on the binding of the basic protein by Nuvia aPrime 4A; **B**, effect of binding buffer pH and NaCl on monomer content in the flow-through fraction; **C**, effect of binding buffer pH and NaCl on monomer recovery in the flow-through fraction.

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Excellent Pressure-Flow Properties

Nuvia aPrime 4A Resin is designed with a bead size optimized to achieve process-scale purification of biomolecules at high flow rates without being limited by column pressure. This provides an increase in productivity. The column pressure remains below 3 bar up to a linear velocity of 350 cm/hr (Figure 4). Nuvia aPrime 4A Resin's fast mass transfer dynamics ensure efficient chromatography at these high flow rates, making it an ideal choice for manufacturing purifications.

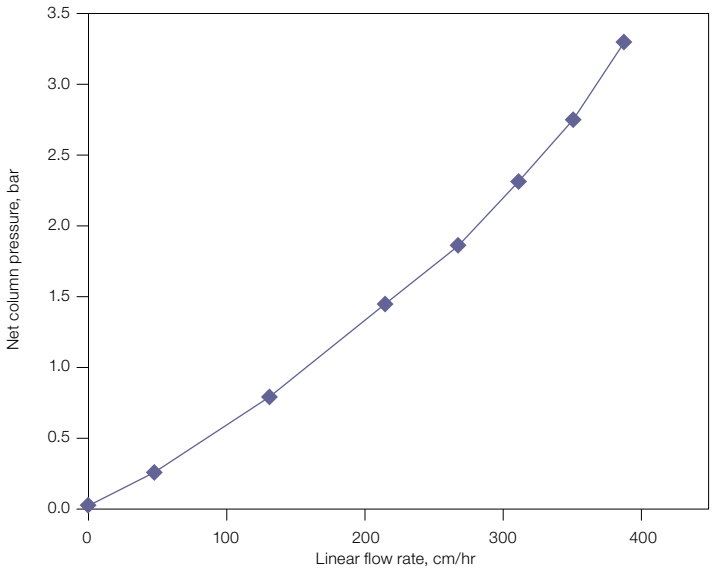

Fig. 4. Pressure-flow performance of Nuvia aPrime 4A Resin. Nuvia aPrime 4A Resin slurry prepared in 1x PBS, pH 7.5 was packed into a 20 x 20 cm column by axial compression at 300 cm/hr with a compression factor of 1.25.

Consistent Binding Capacity at Fast Flow Rates

Nuvia aPrime 4A Resin is built to have superior mechanical strength and optimized pore structure to ensure that the DBC is maintained at the high flow rates required for process purification (Figure 5). This ensures that it delivers the productivity needed in downstream manufacturing.

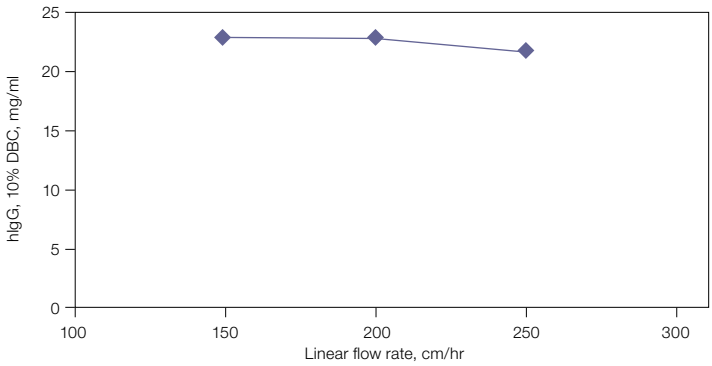

Fig. 5. Effect of flow rate on Nuvia aPrime 4A Resin binding capacity for polyclonal human IgG (hIgG). Bio-Scale MT10 Column dimension: 1.2 x 8.8 cm; equilibration buffer: 50 mM sodium phosphate, pH 8.0. DBC, dynamic binding capacity.

Robust Performance and Recovery

Nuvia aPrime 4A is built on a mechanically stable base bead. It is produced by a validated manufacturing process, which ensures batch-to-batch reproducibility. The chemical stability of Nuvia aPrime 4A allows the resin to perform consistently with minimal changes to dynamic binding capacity or recovery even after multiple exposures to NaOH (Figure 6).

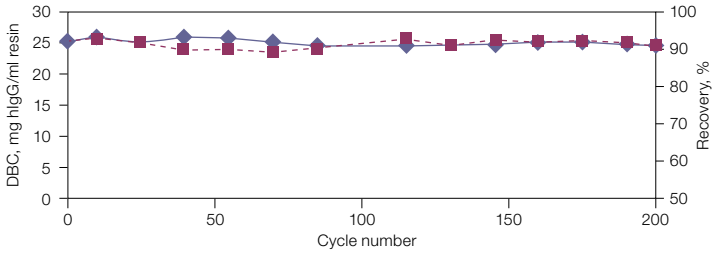

Fig. 6. Stability, reusability, and recovery with Nuvia aPrime 4A Resin. Polyclonal hIgG (1.0 mg/ml) in equilibration buffer (50 mM Na_2PO_4, pH 8.0) was loaded onto a 1 ml Bio-Scale Mini Column (0.56 x 4 cm) packed with Nuvia aPrime 4A. The column was operated at 150 cm/hr. hIgG was eluted with 100 mM sodium citrate, pH 3.0. The column was cleaned in place for 30 min with 1 M NaOH. The 10% DBC and recovery of hIgG at linear velocity of 150 cm/hr was determined after every ten cycles. DBC (—◆—); recovery (---■---). DBC, dynamic binding capacity; hIgG, human IgG.

Easy Scalability from Laboratory to Bioprocess Manufacturing

Nuvia aPrime 4A is specifically designed for easy scalability to meet manufacturing demands. It is available in multiple user-friendly formats, including prepacked Foresight Columns and Plates for purification condition screening and bulk bottles for pilot- to manufacturing-scale purifications. It is backed by our regulatory support documentation and security of supply commitment.

Screen this resin for your application.
Visit bio-rad.com/ResinSample to request a sample.

Technical Assistance

A regulatory support file is available upon request.
Bio-Rad Laboratories, Inc. is an ISO 13485 registered corporation.

Visit bio-rad.com/NuviaaPrime4A for details about this resin.

Visit bio-rad.com/ProcessResins for more information about Bio-Rad's complete line of process chromatography supports.

For additional information and technical assistance, contact your local Bio-Rad office or email our process specialists at **process@bio-rad.com**. In the U.S. and Canada, call 1-800-4BIORAD (1-800-424-6723).

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Ordering Information

Catalog #	Description
12007397	**Nuvia aPrime 4A Resin**, 25 ml
12007396	**Nuvia aPrime 4A Resin**, 100 ml
12007379	**Nuvia aPrime 4A Resin**, 500 ml
12007380	**Nuvia aPrime 4A Resin**, 5 L
12007391	**Nuvia aPrime 4A Resin**, 10 L
12007411	**Foresight Nuvia aPrime 4A Plates**, 2 x 96-well, 20 µl
12007392	**Foresight Nuvia aPrime 4A Column**, 1 ml
12007393	**Foresight Nuvia aPrime 4A Column**, 5 ml
12007394	**Foresight Nuvia aPrime 4A RoboColumn Unit**, 200 µl
12007395	**Foresight Nuvia aPrime 4A RoboColumn Unit**, 600 µl

Larger volumes and special packaging for industrial applications are available upon request.

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