Nuvia aPrime 4A Hydrophobic Anion Exchange Resin

Instruction Manual

Catalog numbers

12007397
12007396
12007379
12007380
12007391
12007411
12007392
12007393
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12007395

Please read the instructions in this manual prior to using Nuvia aPrime 4A Hydrophobic Anion Exchange Resin. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Table of Contents

Section 1
Introduction

Nuvia aPrime 4A Hydrophobic Anion Exchange Resin is a new addition to Bio-Rad's existing family of mixed-mode resins, which includes Nuvia cPrime Hydrophobic Cation Exchange Resin and the CHT family of ceramic hydroxyapatites. It is designed with a positively charged hydrophobic ligand on the surface of the high-performance UNOsphere diol base bead. Nuvia aPrime 4A Resin can function effectively across a wide range of salt concentrations and pH, making it suitable for easy integration into a multistep process. The resin's ligand density and hydrophobicity are designed to facilitate selective and readily reversible binding of target molecules for maximal purity and recovery. The ligand design permits straightforward purification method development.

Nuvia aPrime 4A Resin can be used in the purification of both established therapeutic proteins and diverse new constructs in development. Salt- and pH-sensitive proteins with a high propensity for aggregation and/or degradation can also be effectively purified on Nuvia aPrime 4A Resin. It is specifically designed for easy scalability to meet manufacturing demands. Nuvia aPrime 4A Resin 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. See the Nuvia aPrime 4A Hydrophobic Anion Exchange Resin Product Information Sheet (bulletin 7142) and Table 1 for more product details.

If you have questions or require methods development assistance with Nuvia aPrime 4A Resin, contact your local Bio-Rad process chromatography representative or the Bio-Rad chromatography technical support group for assistance at 1-800-424-6723.

Section 2
Technical Description

Table 1. Nuvia aPrime 4A Resin technical description.

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 (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.

Section 3
Preparation

Nuvia aPrime 4A Resin is supplied fully hydrated in 20% ethanol + 1 M NaCl as a 50% (v/v) slurry. For column packing, replacing the shipping solution with packing buffer is recommended. Small volumes of Nuvia aPrime 4A Resin can be easily washed in a Buchner funnel with 4–5 bed volumes of water or buffer. For large volumes, cycling through 3–4 settling and decanting steps using the packing buffer in the shipping container is recommended.

Removal of fines from Nuvia aPrime 4A Resin is not required. If, however, particle fines have been generated during handling, resuspend the settled resin and remove any opaque or cloudy supernatant before resettling is complete. Repeat until supernatant is clear.

Section 4
Column Packing

Nuvia aPrime 4A Resin can be packed using standard column packing methods. To pack columns for optimal operation, a 20–50% slurry volume is recommended.

Packing Small Columns

This slurry packing method was designed to pack Nuvia aPrime 4A Resin in a conventional laboratory-scale column with an internal diameter of 5–50 mm. Either a column with a working height of at least twice the bed height or a shorter column with a packing reservoir should be used.

1. Prepare buffered 1x PBS, referred to herein as the packing buffer.
2. Decant the shipping solution away from the resin bed as outlined in section 3, maintaining an approximate slurry percentage of 50%.
3. After thorough buffer exchange, transfer an aliquot of the well-mixed slurry into a graduated cylinder to determine the slurry percentage.
4. Allow the slurry to fully settle in the cylinder. Use the settled bed volume to calculate the v/v resin percentage in the slurry.
5. Using a compression factor of 1.2, calculate the volume of slurry required for the intended bed height.
    a. For example, for a 20 cm bed height in a 3 cm diameter column using a 50% slurry, the volume would be:
    $1.2 \times (20/50\%) \times \pi r^2 = 1.2 \times 40 \times 3.14 \times (1.5)^2 = 339$ ml
6. Add a small amount of packing buffer to the column to wet the bottom frit, then pour in the calculated amount of resin slurry.
7. Insert the column flow adaptor and flow pack at a linear velocity of at least 150 cm/hr with packing buffer for at least 10 min. Stop the flow and adjust the flow adaptor to compress the bed to the intended bed height.
8. Equilibrate with at least 3 column volumes (CV) of equilibration buffer and evaluate column efficiency using your standard operating procedures or the procedure described in section 5.

Packing Process-Scale Columns

1. After removing the storage buffer (section 3), prepare a 20–50% slurry (v/v) with packing buffer (see Table 2). For most process columns, follow the manufacturer's recommendations with one major exception: do not recirculate the Nuvia aPrime 4A Resin slurry through the packing pump.
2. Use a low-shear hydrofoil impeller for automatic mixing or a plastic paddle for manual mixing to avoid damaging the resin. The best overall performance of Nuvia aPrime 4A Resin will be obtained with a compression factor of 1.10–1.25. Compression factor is defined as settled bed height divided by packed bed height.

3. After achieving the desired compression, condition the column by flowing fresh packing or equilibration buffer for 3 CV upflow followed by 3 CV in downflow at the chosen process flow rate. After this flow conditioning step, evaluate column efficiency using your standard operating procedures or the procedure described in section 5.

Detailed packing procedures for process-scale columns can be obtained by contacting your Bio-Rad representative.

Flow Properties

Flow properties are shown in Figures 1 and 2.

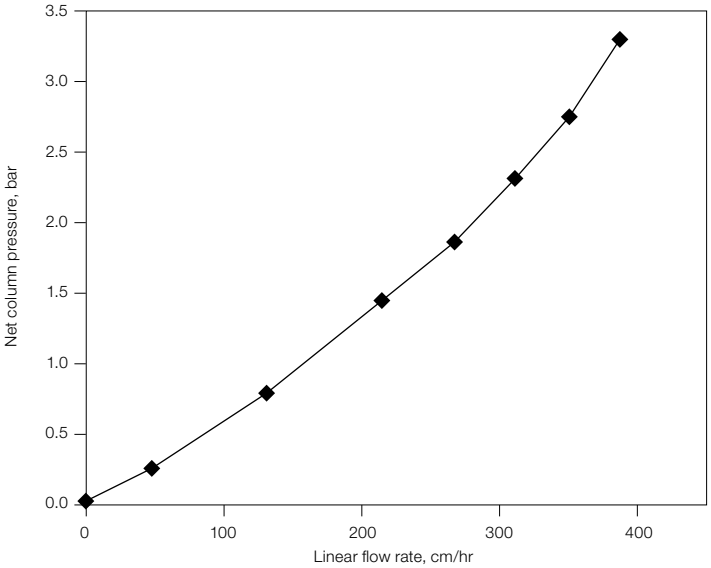


Fig. 1. Pressure-flow performance of Nuvia aPrime 4A Resin. Nuvia aPrime 4A Resin slurry prepared in 1x PBS, pH 7.5 was packed into a 20 x 20 cm column by axial compression at 300 cm/hr with a compression factor of 1.25.

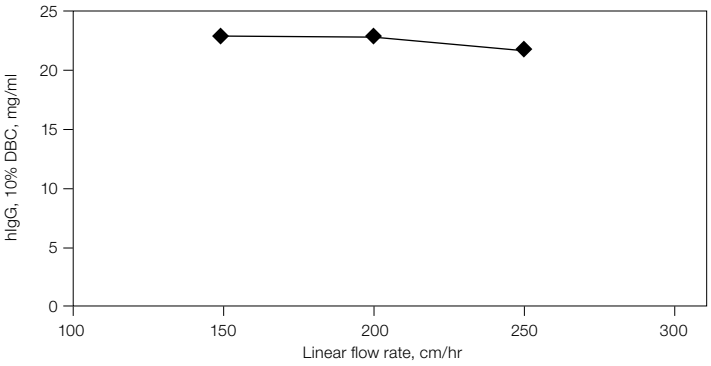


Fig. 2. Effect of flow rate on Nuvia aPrime 4A Resin binding capacity for polyclonal human IgG (hIgG). Bio-Scale MT10 Column dimension: 1.2 x 8.8 cm; equilibration buffer: 50 mM sodium phosphate, pH 8.0. DBC, dynamic binding capacity.

Buffers

All buffers commonly used for anion exchange chromatography can be used with Nuvia aPrime 4A Resin.

Table 2. Common buffers for anion exchange chromatography.

Buffer	Buffering Range, pH
Bicine	7.6–9.0
Bis-Tris	5.8–7.2
Diethanolamine	8.4–8.8
Diethylamine	9.5–11.5
Imidazole	6.6–7.1
L-histidine	5.5–6.0
Pyridine	4.9–5.6
Tricine	7.4–8.8
Triethanolamine	7.3–8.3
Tris	7.5–8.0

Section 5
Column Packing Evaluation

Poor column packing can lead to compromised product quality and economics. Therefore, the efficiency of packing must be tested after each column packing. In addition, packing analysis during process development can assist in setting appropriate acceptance criteria during scale-up.

After column packing is complete, thoroughly equilibrate the column with equilibration buffer. To test the efficiency of the column packing operation, inject a sample of a low molecular weight, unretained compound (for example, 10 mM NaPi in 1 M NaCl) to determine height equivalent to a theoretical plate (HETP). The recommended sample volume is 1–2% of the total column volume. Column testing can be operated at 100 cm/hr.

HETP = L/N
where
L = Bed height (cm)
N = Number of theoretical plates
Calculation for $N = 5.54(V_e/W_{½h})^2$
where
V_e = Peak elution volume or time
$W_{½h}$ = Peak width at peak half height in volume or time
V_e and $W_{½h}$ should always be in the same units.

Reduced plate height can also be used to evaluate column packing efficiency. The reduced plate height h is calculated as follows:
h = HETP/d where d is the diameter of the beads

Peak asymmetry factor calculation:
$A_s = b/a$
a = Front section of peak width at 10% of peak height bisected by line denoting V_e
b = Latter section of peak width at 10% of peak height bisected by line denoting V_e
Peaks should be symmetrical and the asymmetry factor as close as possible to 1.
A_s values of 0.8–1.8 are acceptable.

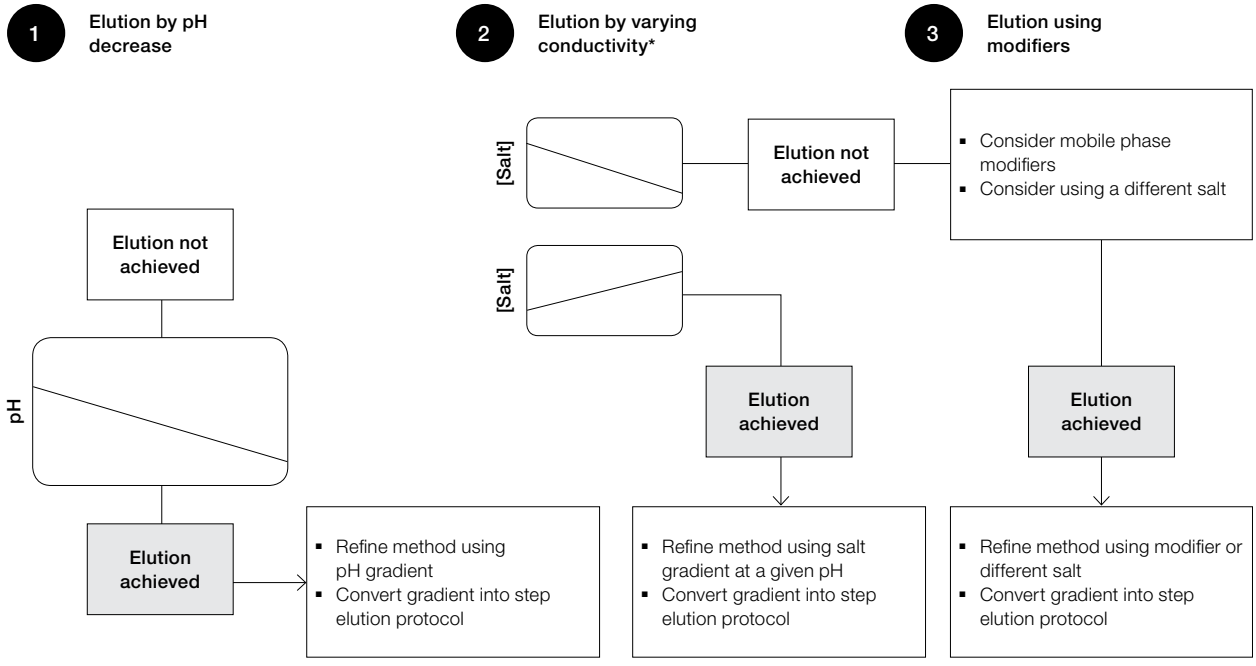
Section 6
Method Development

Developing an effective and robust method with Nuvia aPrime 4A Resin is straightforward. Following is general information on the binding and elution mechanism and an approach to guide method development; results will vary depending on protein of interest and feed composition.

The binding and elution mechanisms of Nuvia aPrime 4A Resin are affected chiefly by buffer pH and salt. The salt tolerance of the resin often allows for direct loading at high conductivity. A decrease in pH will in many cases achieve elution. Changes in ionic strength can also achieve and/or optimize elution and the final method is often a combination of a decrease in pH and/or an increase/decrease in salt concentration. In some cases, the use of an elution buffer modifier or a different salt in the elution buffer may be required for optimal elution, recovery, and resolution.

The schematic below outlines a general method development rationale. In most cases, conducting a few simple design of experiments to identify optimal binding and elution conditions will yield an effective, robust, and scalable method.

General Approach to Method Development



* At optimum pH, determined from step 1.

1. Load feed or eluate from previous step directly or with dilution as needed onto the Nuvia aPrime 4A Resin Column. To elute, use a decreasing pH gradient. If satisfactory elution and recovery are achieved, refine the gradient or convert to step elution.
2. If elution is not satisfactory after step 1, run an increasing or decreasing salt gradient to disrupt electrostatic or hydrophobic interactions, respectively, that may be preventing elution or broadening the peak. Use the pH where there was best elution (from step 1). The final direction of this salt gradient (increasing or decreasing) can be easily assessed and will depend on the relative contributions of ionic vs. hydrophobic interactions involved in binding.
3. If elution is still unsatisfactory after step 2 of this process, consider using a modifier such as propylene glycol, urea, or arginine to disrupt any remaining interactions. Other modifiers may also be used; in some cases changing to another salt may also be required.

For further assistance or to discuss method development, contact the Bio-Rad chromatography technical support group at 1-800-424-6723.

Section 7
Sanitization and Regeneration

After each chromatography run, the packed resin bed should be washed to remove reversibly bound material and prepare the column for the subsequent run. This cleaning process is achieved by washing the column with 2–6 CV of 1–2 M NaCl followed by 2–6 CV of 1 M NaOH to remove remaining proteinaceous impurities. Washing should be conducted until absorbance returns to baseline. The column is now ready to be sanitized in 1 M NaOH. Approximately 50–100 mM sodium acetate (pH 3) can also be used for the removal of remaining impurities if necessary.

To equilibrate the column after sanitization, we recommend applying 4–6 CV of a solution such as 1 M NaCl or equilibration buffer.

If a column no longer yields reproducible results, the resin may require additional cleaning to remove strongly bound contaminants. Acceptable cleaning agents include 25% acetic acid, 8 M urea, 1% Triton X-100, 70% ethanol, 30% isopropyl alcohol, and 6 M guanidine hydrochloride.

Section 8
Storage

Nuvia aPrime 4A Resin is stable at room temperature across a broad pH range (2–14). The medium may be stored in 20% ethanol.

Section 9
Regulatory Support

A regulatory support file is available for Nuvia aPrime 4A Resin. If you need assistance validating the use of Nuvia aPrime 4A Resin in a production process, contact your local Bio-Rad representative.

Section 10
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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