Nuvia™ HR-S Media, a Strong Cation Exchange Resin

Instruction Manual

Catalog #

1560511
1560513
1560515
12018125
1560517
12003714
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12003715
7324723
7324743
7324831
7324832
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Please read these instructions before you use Nuvia HR-S Media. If you have any questions or comments regarding these instructions, please contact your Bio-Rad Laboratories representative.

Visit **bio-rad.com/NuviaHRS** for more information.

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Section 1: Introduction

Nuvia HR-S Media is a high-resolution strong cation exchange chromatography resin in the Nuvia family of ion-exchange resins. The resin is designed for intermediate and final polishing purification steps where separating closely related product impurities (that is, charged variants) is a challenge. Its exceptional selectivity and high recovery meet the biomolecule purification needs at both laboratory and large bioprocess scales.

Nuvia HR-S Media joins the family of Nuvia S, Nuvia Q, and Nuvia HP-Q ion exchange media and may be used for the separation of proteins, nucleic acids, viruses, plasmids, and other macromolecules. The unique properties of Nuvia HR-S Media, with its smaller particle size and fast mass transfer, allow the media to be effective for the resolution of various biomolecules.

Nuvia HR-S Media 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 HR-S Cation Exchange Resin Product Information Sheet (bulletin 6448) for more product details.

If you have questions or require methods development assistance with Nuvia HR-S Media, contact your local Bio-Rad chromatography representative, reach us online at **bio-rad.com/ProcessHelp**, or email us at **process@bio-rad.com**.

Section 2: Technical Description

The technical properties of Nuvia HR-S Media are included in Table 1.

Table 1. Characteristics of Nuvia HR-S Media.

Property	Description
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Base matrix composition	Polyacrylamide
Mean particle size	50 ± 10 µm
Total ionic capacity	100–180 µeq/mL
Dynamic binding capacity*	≥70 mg/mL at 300 cm/hr
Recommended linear flow rate	50–200 cm/hr
Compression factor (settled bed volume/packed bed volume)	1.25
pH stability**	Short term: 2–14 Long term: 4–13
Chemical stability	1.0 M NaOH (20°C): up to 5 weeks 0.1 M NaOH (20°C): up to 5 years
Shipping solution	20% ethanol + 0.1 M NaCl or 2% benzyl alcohol
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 M NaOH
Storage conditions	20% ethanol or 0.1 M NaOH
Storage temperature	Room temperature
Shelf life	5 years

* 10% breakthrough capacity determined with 5.0 mg/mL human IgG in 20 mM NaOAc, pH 5.0.

** Data derived under accelerated conditions at 60°C.

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Section 3: Preparation

Nuvia HR-S Media is supplied fully hydrated as 50% (v/v) slurry in 20% ethanol + 0.1 M NaCl or 2% benzyl alcohol. For column packing, replacing the shipping solution with packing buffer is recommended. Small volumes of Nuvia HR-S Media are easily washed in a Büchner funnel with 4–5 volumes of packing buffer. For large volumes, cycling through 3–4 settling and decanting steps using the packing buffer is recommended.

Removal of fines from Nuvia HR-S Media is not required due to the narrow particle-size range. If fines have been generated during handling, resuspend the slurry and remove the opaque supernatant before sedimentation is complete. Repeat several times.

Section 4: Column Packing

Nuvia HR-S Media can be packed using most conventional packing methods. A slurry concentration of 20–50% is recommended. The compression factor for Nuvia HR-S Media is 1.25. The compression factor is defined as gravity-settled bed height divided by packed bed height. The packing factor is defined as the consolidated bed height by packed bed height. Axial, flow, or hybrid (a combination of axial and flow) consolidation methods are recommended at a linear velocity of 50 to 200 cm/hr.

Packing Laboratory-Scale Columns

This slurry packing method was designed to pack Nuvia HR-S Media in a conventional column with an internal diameter of 5–15 mm. All buffers should be degassed. Either a column with a working height at least twice the bed height or a shorter column with a packing reservoir should be used.

1. Prepare degassed 0.1 M NaCl and 20–50 mM buffer salt, hereafter referred to 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 1.25, calculate the volume of slurry required for the intended bed height. For example, for a 20 cm bed height in a 3 cm diameter column using a 50% slurry, the volume would be: $1.25 \times (20/50\%) \times \pi r^2 = 1.25 \times 40 \times 3.14 \times (1.5)^2 = 353$ 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 50–200 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.

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Packing Process-Scale Columns

1. After removing the shipping buffer (Section 3), prepare a 20–50% slurry (v/v) with packing buffer. Follow the column manufacturer's recommendations with one major exception: do not recirculate the Nuvia HR-S Media slurry through the packing pump.
2. Use a low-shear hydrofoil impeller for automated mixing or a plastic paddle for manual mixing. The best overall performance of Nuvia HR-S Media will be obtained with a compression factor of 1.25, defined as the settled bed height divided by the packed bed height.
3. Accurate determination of slurry percentage is essential for packing process-scale columns. The Econo-Pac Chromatography Column (Bio-Rad Laboratories, Inc., catalog #7321010, package of 50) may be used to quickly determine slurry percentage as follows.

   First, after re-slurrying and combining equilibrated resin in the packing buffer required for the column, pour a volume of resin into no less than three bottom-capped columns (new Econo-Pac columns are pre-capped, or a reusable End Caps, Bio-Rad #7311660, may be used on previously used columns) and mark each column at the slurry height.

   Next, uncap the bottom of each column and allow the columns to drain completely. Because this resin will not be used in a process, it is acceptable for the packing buffer to drain out of each column. Once each column has drained, measure the height of the resulting resin bed from the top of each column's bottom frit, and measure the height of the mark (indicating the height of the slurry added) from the same reference point (top of each column's bottom frit).

   To calculate slurry percentage, divide the resin bed height by the slurry height, multiply by 100 for each column, and average the results. Slurry percentage should be between 50–70% for optimal packing.
4. Bio-Rad recommends using process columns with axial compression capabilities for best results. For stall-packing type columns, additional compression may be required to achieve a compression factor of 1.25. A linear velocity of 50–200 cm/hr is recommended. Packing factors listed in Table 2 are recommended in a 0.1 M NaCl system to achieve a compression factor of 1.25.

Table 2. Starting packing factor recommendations in 0.1 M NaCl.

Linear Flow Rate, cm/hr	Packing Factor
50	1.17
60	1.15
75	1.14
100	1.12
125	1.1
150	1.08
175	1.06
200	1.04

5. After the desired compression is achieved, flow condition the column with fresh packing or equilibration buffer for 3 CV in upflow, followed by 3 CV in downflow at the process flow rate. After flow conditioning, 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 local Bio-Rad representative.

Section 5: Column Packing Evaluation

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

To determine asymmetry and plate count (HETP), thoroughly equilibrate the column in packing or other buffer. Inject a small amount (1–2% of the total column volume) of that buffer containing a low molecular weight, unretained compound (e.g., by adding acetone or 1 M NaCl) and monitor the column effluent appropriately.

If acetone is the test marker (using an ultraviolet absorbance monitor at 280 nm), the equilibration buffer must have a salt concentration of less than 100 mM. If 1 M NaCl is the test marker (using a conductivity monitor), then the equilibration buffer salt concentration should be 100–200 mM. Typical flow rates for this procedure are 100–150 cm/hr. Be aware that flow rate can significantly affect plate count, as governed by the Van Deemter equation (DOI: 10.1016/0009-2509(56)80003-1).

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Height equivalent to a theoretical plate (HETP)

HETP is calculated as:

HETP = L/N

where:

L = bed height (cm)

N = number of theoretical plates

N is calculated as:

$N = 5.54 (V_e/W_{1/2h})^2$

where:

V_e = peak elution volume or time

$W_{1/2h}$ = peak width at peak half height in volume or time

V_e and $W_{1/2h}$ should always be in the same units.

Reduced plate height (h)

h can also be used to evaluate column packing efficiency. It is calculated as follows:

h = HETP/d

where:

d = bead diameter

Peak asymmetry factor (A_s)

A_s = b/a

where:

a = front section of peak width at 10% of peak height, bisected by the line denoting V_e

b = latter section of peak width at 10% of peak height, bisected by the line denoting V_e

Peaks should be symmetrical, and the asymmetry factor should be as close as possible to 1.

A_s values of 0.8–2.5 are acceptable.

Section 6: Operation and Maintenance

All Nuvia Resins are designed to achieve the highest productivity (grams of drug per operational hour per liter of media) possible. Nuvia Resins should be run at the highest linear velocities and loading capacities allowed by the column and the chromatography system.

A linear flow rate of 100 cm/hr with a 20 cm bed is a recommended starting point for Nuvia HR-S Media. Purification may be optimized by changing the pH, flow rate, or ionic strength of the elution buffer, modifying the gradient profile, or experimenting with different buffer salts.

Note: Due to the smaller particle size of Nuvia HR-S Media, it is recommended to run the process at flow rates no greater than 200 cm/hr to minimize backpressure and headspace formation.

Figure 1 shows the effect of flow rate on backpressure.

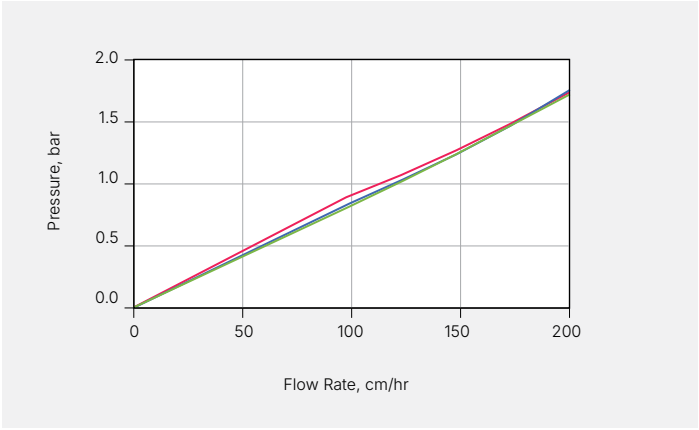

Fig. 1. Pressure-flow performance of Nuvia HR-S Media at a compression factor of 1.25. Data are shown for a 45 cm internal diameter (ID) column (—) and 20 cm ID columns from Manufacturer A (—) and Manufacturer B (—).

All buffers commonly used for ion exchange chromatography can be used with HR-S Media (see Table 3). Buffering ions that have the same charge as the functional group on the ion exchanger will produce the best results.

Table 3. Common buffers for ion exchange chromatography.

Buffer	Buffering Range
Acetic acid	4.8–5.2
Citric acid	4.2–5.2
HEPES	6.8–8.2
Lactic acid	3.6–4.3
MES	5.5–6.7
MOPS	6.5–7.9
Phosphate	6.7–7.6
PIPES	6.1–7.5
TES	6.8–8.2
Tricine	7.8–8.9

Section 7: Regeneration and Sanitization

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 can then be sanitized in 1.0 N NaOH at 50–100 cm/hr; a minimum contact time of 120 min is recommended.

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

Section 8: Cleaning in Place (CIP) and Sanitization

If a column no longer yields reproducible results, the media may require thorough CIP and sanitization after regeneration to remove strongly bound contaminants. Acceptable CIP agents include 25% acetic acid, 8 M urea, 1% octylphenol ethoxylate, 6 M potassium thiocyanate, 70% ethanol, 30% isopropyl alcohol, 1 N NaOH, 6 M guanidine hydrochloride, and 1 to 5% SDS.

Section 9: Storage

Nuvia HR-S Media is stable at room temperature across a broad pH range (4–13). The resin may be stored in either 2% benzyl alcohol or 20% ethanol. For long-term storage, Nuvia HR-S Media must be equilibrated with 0.01–0.1 N NaOH, 20% ethanol, or 2% benzyl alcohol.

Section 10: Regulatory Support

A regulatory support file is available for Nuvia HR-S Media. For assistance validating the use of Nuvia HR-S Media in a production process, contact your Bio-Rad chromatography representative, reach us online at **bio-rad.com/ProcessHelp**, or email us at **process@bio-rad.com**.

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Section 11:

Ordering Information

Catalog #	Description
1560511	Nuvia HR-S Media, 25 ml
1560513	Nuvia HR-S Media, 100 mL
1560515	Nuvia HR-S Media, 500 mL
12018125	Nuvia HR-S Media, 5 L
1560517	Nuvia HR-S Media, 10 L
12003714	Nuvia HR-S Media, 500 mL (in BA)
12018182	Nuvia HR-S Media, 5 L (in BA)
12003715	Nuvia HR-S Media, 10 L (in BA)
7324723	Foresight Nuvia HR-S Column, 1 mL
7324743	Foresight Nuvia HR-S Column, 5 mL
7324831	Foresight Nuvia HR-S RoboColumn Unit, 200 µL
7324832	Foresight Nuvia HR-S RoboColumn Unit, 600 µL
7324707	Foresight Nuvia HR-S Plates, 20 µL

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

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

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