Nuvia™ HR-S Strong Cation Exchange Media

Instruction Manual

Catalog numbers

156-0511
156-0513
156-0515
156-0517

Please read these instructions before you use Nuvia HR-S cation exchange media. If you have any questions or comments regarding these instructions, please contact your Bio-Rad Laboratories representative.

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Section 1: Introduction

Nuvia™ HR-S cation exchange media is a new high-resolution chromatography media 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 scale.

Nuvia HR-S media joins the family of Nuvia S and Nuvia 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.

If you have questions about Nuvia media, contact either your local Bio-Rad process chromatography sales representative or the Bio-Rad chromatography Technical Support department for further assistance at 1-510-741-6563.

Section 2: Technical Description

Table 1. Characteristics of Nuvia HR-S media.

	Nuvia HR-S
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Total ionic capacity	100–180 µeq/ml
Dynamic binding capacity*	≥70 mg/ml at 300 cm/hr
Shipping counterion	Na^+
Median particle size	50 ± 10 µm
Recommended linear flow rate range	50–200 cm/hr
Chemical stability	–
1.0 N NaOH (20°C)	up to 5 weeks
0.1 N NaOH (20°C)**	up to 5 years
Compression factor	1.25 (settled bed volume/packed bed volume)
pH stability**	2–14 short term 4–13 long term
Shipping solution	20% ethanol
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 N NaOH

* 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.

Section 3: Preparation

Nuvia HR-S media is supplied fully hydrated in 20% ethanol + 1 M NaCl as a 50% (v/v) slurry. For column packing, removal of the shipping 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 until supernatant is clear.

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 is 1.25. The compression factor is defined as settled bed height divided by packed bed height.

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. Because a relatively large volume of slurry is required, it is recommended that a packing reservoir be used.

1. Prepare degassed 1.0 M NaCl, 20–50 mM buffer salt (see Table 2), referred to hereunder as the packing buffer.
2. Nuvia media are shipped as a 50% slurry. Measure the desired amount of suspended slurry into a graduated cylinder. Allow the resin bed to settle. Decant the shipping solution from the settled media.
3. Add sufficient degassed packing buffer to the resin to make a 20–50% slurry.
4. Mix to suspend the resin. Caution: Do not mix with a magnetic stir bar as damage may occur. Larger amounts of slurry may be mixed with a low-shear marine impeller at low to moderate speed.
5. Add packing buffer to the column to about 2–5 cm high. Pour in prepared resin slurry.
6. Once a layer of supernatant has developed, insert the column flow adaptor and flow pack at a linear velocity of 300–600 cm/hr with packing buffer for at least 10 min. Lower the adaptor to the calculated compressed bed height.

Packing Process-Scale Columns

After removing the storage buffer (Section 3), prepare a 20–50% slurry (v/v) with packing buffer (see Table 2). Follow the column manufacturer's recommendations with one major exception: do not recirculate the Nuvia slurry through the packing pump.

Use a low-shear hydrofoil impeller for automated mixing or a plastic paddle for manual mixing. The best overall performance of Nuvia HR-S will be obtained with a compression factor of 1.25, defined as settled bed height divided by the packed bed height.

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.

After the desired compression is achieved, flow condition the column with fresh packing or equilibration buffer for 3 column volumes (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 Bio-Rad representative.

Section 5: Column Packing Evaluation

When column packing is complete, equilibrate the column until baseline conductivity is stable. To test the effectiveness of column packing, inject a sample of a low molecular weight, unretained compound (for example, acetone or 1 M NaCl). If acetone is used as the test marker (use a UV absorbance monitor set at 280 nm), the equilibration buffer must have a salt concentration <100 mM. If 1 M NaCl is the test marker (use a conductivity monitor), then the equilibration buffer salt concentration should be 100–200 mM. The sample volume should be 1–3% of the total column volume. Column testing should be operated using the same linear velocity used to load and/or elute the sample.

To obtain comparable height equivalent to a theoretical plate (HETP) values among columns, the same conditions must be applied.

N = Number of theoretical plates

$N = 5.54(V_e/W_{½h})^2$

L = Bed height, cm

HETP = L/N

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

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

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

A_s equal to 0.8–2.5 should be easily achieved under normal operating conditions.

Section 6: Operation and Maintenance

A linear flow rate of 100 cm/hr with a 20 cm bed is a recommended starting point for Nuvia HR-S. 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.

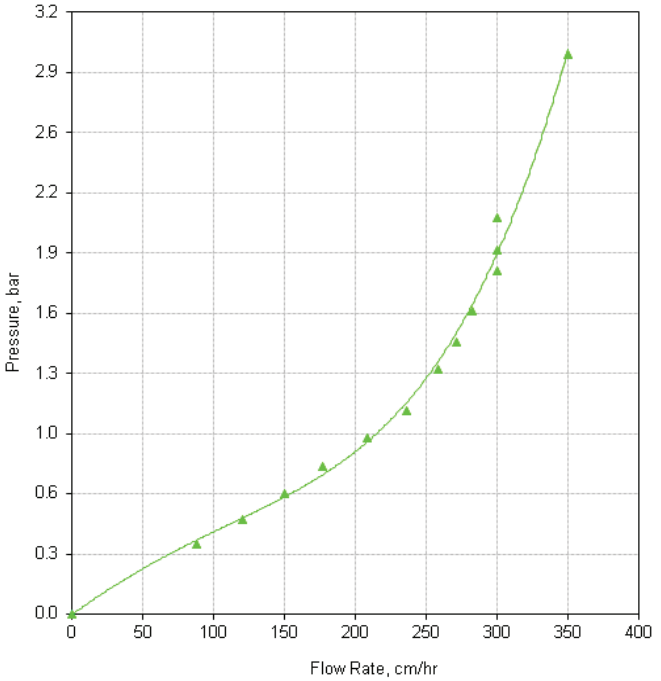

Fig. 1. Nuvia HR-S media pressure/flow performance for a 20 cm diameter x 20 cm bed height column packed to a compression factor of 1.25.

All buffers commonly used for ion exchange chromatography can be used with Nuvia media (see Table 2). Buffering ions that have the same charge as the functional group on the ion exchanger will produce the best results.

Table 2. 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 run, the packed bed should be washed with 2–6 bed volumes of 1–2 M NaCl or until absorbance returns to baseline to remove reversibly bound material. 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.

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% Triton X-100, 6 M potassium thiocyanate, 70% ethanol, 30% isopropyl alcohol, 1 N NaOH, 6 M guanidine hydrochloride, and 1 to 5% SDS.

Section 9: Storage

For long-term storage, Nuvia HR-S media should be equilibrated with 0.01–0.1 N NaOH or 20% ethanol (see Table 1).

Section 10: Regulatory Support

Regulatory support files are available for Nuvia HR-S media. If you need assistance validating the use of Nuvia media in a production process, contact your local Bio-Rad representative.

Section 11: Ordering Information

Catalog #	Description
156-0511	**Nuvia HR-S Media**, 25 ml
156-0513	**Nuvia HR-S Media**, 100 ml
156-0515	**Nuvia HR-S Media**, 500 ml
156-0517	**Nuvia HR-S Media**, 10 L
732-4707	**Foresight™ Nuvia™ HR-S Plates**, 20 µl
732-4831	**Foresight Nuvia HR-S RoboColumn Units**, 200 µl
732-4832	**Foresight Nuvia HR-S RoboColumn Units**, 600 µl
732-4723	**Foresight Nuvia HR-S Column**, 1 ml
732-4743	**Foresight Nuvia HR-S Column**, 5 ml

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

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