Nuvia HP-Q Strong Anion Exchange Media

Instruction Manual

Catalog numbers

12006693
12006691
12006660
12006659
12007023
12007022
12007018
12007019
12007033
12006994
12007020
12007021
12007013
12007014
12006908

Please read these instructions prior to using the Bio-Rad Nuvia HP-Q Resin. If you have any questions or comments regarding these instructions, contact your Bio-Rad Laboratories representative.

BIO-RAD

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Section 1
Introduction

Nuvia HP-Q is a high-performance strong anion exchange resin. It is the latest product in the Nuvia family of high-capacity ion-exchange resins and can be used for downstream purification of large molecules, such as high molecular weight (HMW) plasma proteins IgA and IgM, viruses, virus-like particles, and PEGylated proteins. It is built on the rugged and hydrophilic UNOsphere epoxide base bead that provides fast mass transfer kinetics and low nonspecific binding, which are demanded by today's process manufacturing. The stability of this base bead and its broad chemical compatibility allow repeated uses with a long resin lifetime. The particle size of Nuvia HP-Q Resin is designed to offer high dynamic binding capacities at fast flow rates without excessive backpressure, thereby delivering excellent process economics. Its pore size is optimized for easy accessibility and adsorption of large biomolecules and the internal spacer length and ligand density facilitate efficient binding of the biomolecules even at high flow rates.

Nuvia HP-Q Resin is compatible with a flexible process design since it works in an operational window with a range of pH and flow rates. It is also compatible with high-throughput purification to improve productivity and process economics.

Nuvia HP-Q Resin 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. See the Nuvia HP-Q Anion Exchange Resin Product Information Sheet (bulletin 7078) for more product details.

Section 2
Technical Description

The technical properties of Nuvia HP-Q Resin are included in Table 1.

Table 1. Nuvia HP-Q Resin technical description.

Property	Description
Type of ion exchanger	Strong anion
Functional group	$-N^{+}(CH_3)_3$
Particle size	38–53 µm
Total ionic capacity	48–88 µeq/ml
Dynamic binding capacity*	>50 mg/ml at 100 cm/hr
Recommended linear flow rate	50–300 cm/hr
Compression factor	~1.2
pH stability	Short-term: 2–13 Long-term: 2–10
Shipping solution	20% ethanol or 2% benzyl alcohol
Regeneration	1–2 M NaCl
Sanitization	1 N NaOH
Storage conditions	20% ethanol or 0.01 N NaOH
Storage temperature	Room temperature
Chemical stability	1 N NaOH (22°C): up to 1 week** 0.01 N NaOH (22ºC): up to 5 weeks
Shelf life	5 years

* 10% breakthrough capacity determined with 1.1 mg/ml of thyroglobulin in 20 mM Tris-HCl, pH 8.0.

** When testing for chemical stability with 1 N NaOH at 22ºC, ionic capacities dropped to 49 µeq/ml at 1 week and below the acceptance criteria of 68 ± 20 µeq/ml thereafter. Other measured properties of Nuvia HP-Q Resin remained unaffected.

Section 3
Preparation

Nuvia HP-Q Resin is supplied fully hydrated as 50% (v/v) slurry in 20% ethanol or 2% benzyl alcohol. For column packing, replacing the shipping solution with packing buffer is recommended. Small volumes of Nuvia HP-Q Resin can be easily washed in a Büchner funnel with 4–5 bed volumes of water or buffer. For large volumes, cycling through three to four settling and decanting steps using the packing buffer in the shipping container is recommended.

Removal of fines from Nuvia HP-Q Resin is not required due to the narrow particle size range. If fines have been generated during handling, resuspend the settled resin and remove the opaque supernatant before sedimentation is complete. Repeat several times.

Section 4
Column Packing

Nuvia HP-Q Resin can be packed using pressure, volumetric flow, or vacuum packing methods. To pack columns for highly efficient operation, a 20–50% slurry volume is recommended.

Packing Small-Scale Columns

This slurry packing method was designed to pack Nuvia HP-Q Resin in a conventional column with an internal diameter of 5–15 mm. All buffers should be degassed. Since a relatively large volume of slurry is required, a packing reservoir should be used.

1. Prepare degassed 1.0 M NaCl in 20–50 mM buffer (see Table 2), referred to herein as the packing buffer.
2. Nuvia HP-Q Resin is shipped as 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 resin.
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 hydrofoil 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 buffer with NaCl for at least 20 min. Lower the adaptor to the calculated compressed bed height.

Packing Process-Scale Columns

After removing the storage buffer (Section 3), prepare 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 automatic mixing or a plastic paddle for manual mixing. Good performance of Nuvia HP-Q Resin will be obtained with a compression factor of 1.20, 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 columns, a resin slurry of lower concentration is recommended.

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 300 cm/hr or the maximum flow rate or pressure allowed (refer to the hardware manufacturer's manual). After flow conditioning, evaluate column efficiency using 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.

After column packing is complete, equilibrate the column with up to 5 CV equilibration buffer. To test the efficiency of the column packing operation, inject a sample of a low molecular weight, unretained compound (for example, acetone or 1 M NaCl) to determine the height equivalent to a theoretical plate (HETP). If acetone is used as the test marker (use an ultraviolet 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 recommended sample volume is 1–3% of the total column volume.

To obtain comparable HETP values among columns, the same conditions must be applied. Minimum theoretical plate values should be 1,000–3,000 plates/m for linear velocities of 50–400 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
Note: V_e and $W_{½h}$ should always be in the same units.

Reduced plate height (h) can also be used to evaluate column packing efficiency.
Reduced plate height calculation:
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
Note: Peaks should be symmetrical and the asymmetry factor as close as possible to 1. A_s = 0.8–1.8 is acceptable.

Section 6
Operation and Maintenance

All the 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 150 cm/hr and a 20 cm bed is a recommended starting point. Purification may be optimized by changing the pH, flow rate, or ionic strength of the buffers, modifying the gradient profile, or experimenting with different buffer salts.

Figure 1 shows the effect of flow rate on backpressure.

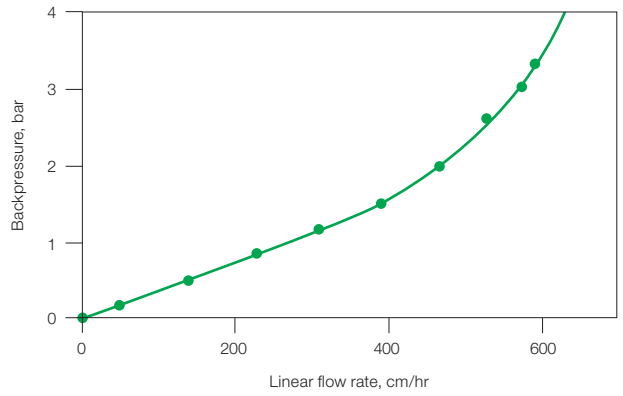

Fig. 1. Pressure and flow performance of Nuvia HP-Q Resin. Nuvia HP-Q Resin slurry prepared in 1x phosphate buffered saline, pH 7.5, was packed into a 20 x 20 cm column by axial compression with a compression factor of 1.20.

All buffers commonly used for anion exchange chromatography can be used with Nuvia HP-Q Resin (Table 2). The use of buffering ions that have the same charge as the functional group on the ion exchanger will produce the best results.

Table 2. Buffers compatible with Nuvia HP-Q Resin.

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 7
Regeneration and Sanitization

After each run, the packed resin 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 be sanitized in 1.0 N NaOH at 50–100 cm/hr.

Section 8
Cleaning in Place (CIP)

If a column no longer yields reproducible results, the resin 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, and 6 M guanidine hydrochloride.

Section 9
Storage

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

Section 10
Regulatory Support

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

Section 11
Ordering Information

Catalog #	Description
12006693	**Nuvia HP-Q Media**, 25 ml
12006691	**Nuvia HP-Q Media**, 100 ml
12006660	**Nuvia HP-Q Media**, 500 ml
12006659	**Nuvia HP-Q Media**, 5 L
12007023	**Nuvia HP-Q Media**, 10 L
12007022	**Nuvia HP-Q Media**, in benzyl alcohol, 25 ml
12007018	**Nuvia HP-Q Media**, in benzyl alcohol, 100 ml
12007019	**Nuvia HP-Q Media**, in benzyl alcohol, 500 ml
12007033	**Nuvia HP-Q Media**, in benzyl alcohol, 5 L
12006994	**Nuvia HP-Q Media**, in benzyl alcohol, 10 L
12007020	**Foresight Nuvia HP-Q Column**, 1 ml
12007021	**Foresight Nuvia HP-Q Column**, 5 ml
12007013	**Foresight Nuvia HP-Q RoboColumn Unit**, 200 µl
12007014	**Foresight Nuvia HP-Q RoboColumn Unit**, 600 µl
12006908	**Foresight Nuvia HP-Q Plates**, 2 x 96-well, 20 µl

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

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

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Life Science Group

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