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# Nuvia™ S and Q High-Capacity Ion Exchange Media

## Instruction Manual

### Catalog numbers

156-0311            156-0411

156-0313            156-0413

156-0315            156-0415

156-0317            156-0417

Please read these instructions before you use Nuvia S and Q High-capacity ion 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™ S and Q high-capacity ion exchange media are new ultra high binding capacity chromatography media designed for any stage in the purification process from capture to final polish. Their robust physical properties, high productivity, and exceptional selectivity meet the needs for biomolecule purification at both laboratory scale and biopharmaceutical process scale. Nuvia media are designed for the separation of proteins, nucleic acids, viruses, plasmids, and other macromolecules. Nuvia media offer high binding capacity at fast linear flow rates while maintaining low backpressure. The unique properties of Nuvia media position them as highly effective media for biomolecule purification.

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™ media.**

	Nuvia S	Nuvia Q
Type of ion exchanger	Strong cation	Strong anion
Functional group	-SO <sub>3</sub> <sup>-</sup>	-N(CH <sub>3</sub> ) <sub>3</sub> <sup>+</sup>
Total ionic capacity	90–150 µeq/ml	100–170 µeq/ml
Dynamic binding capacity*	300 cm/hr ≥110 mg/ml	300 cm/hr >170 mg/ml
Shipping counterion	Na <sup>+</sup>	Cl <sup>-</sup>
Median particle size	85 ± 15 µm	85 ± 15 µm
Recommended linear flow rate range	50–600 cm/hr	50–600 cm/hr
Chemical stability		
1.0 N NaOH (20°C)	up to 1 week	up to 1 week
0.1 N NaOH (20°C)**	up to 5 yr	—
0.01 N NaOH (20°C)**	—	up to 5 yr
Gel bed compression ratio	1.15–1.18 (settled bed volume/packed bed volume)	1.10–1.15
pH stability**	2–14 short term 4–13 long term	2–14 short term 4–12 long term
Shipping solution	20% ethanol + 0.1 M NaCl	20% ethanol + 1 M NaCl
Regeneration	1–2 M NaCl	1–2 M NaCl
Sanitation	0.5–1.0 N NaOH	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 N NaOH	20% ethanol or 0.01 N NaOH

\* 10% breakthrough capacity determined with 4.5 mg/ml human IgG in 40 mM NaOAc + 30 mM NaCl, pH 5.0 for Nuvia S and 5 mg/ml BSA in 20 mM Tris-HCl pH 8.5 for Nuvia Q.

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

NOTE: Recommended elution flow rates are ≤150 cm/hr to minimize viscosity induced backpressure.

## Section 3: Preparation

Nuvia™ media are 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 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 in the shipping container is recommended.

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

## Section 4: Column Packing

Nuvia™ media can be packed using pressure, volumetric flow, or vacuum packing methods. To pack columns for highly efficient operation, it is recommended that a 20–50% slurry volume be used.

### Packing Small Columns

This slurry packing method was designed to pack 25 ml of Nuvia 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 herein as the packing buffer.
2. Nuvia media are shipped as a 50% slurry. Measure 50 ml of suspended slurry into a 100 ml graduated cylinder. Allow the resin bed to settle. Decant the shipping solution away from the resin bed.
3. Add 50 ml degassed packing buffer to the resin.
4. Seal the cylinder and rotate it 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 10 ml packing buffer to the column. Pour in 75 ml resin slurry.
6. 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. Note the compressed bed height, stop the flow, and adjust the flow adaptor to compress the bed 0.1–1.0 cm.
7. Attach the column to your chromatography system, and purge the column with starting buffer at linear velocities up to 600 cm/hr. If the bed compresses, repeat steps 6 and 7.

## 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 impeller for automatic mixing or a plastic paddle for manual mixing. The best overall performance of Nuvia S and Nuvia Q will be obtained with a compression ratio of 1.15–1.20 and 1.10–1.15 respectively defined as settled bed height /packed bed height. After the desired compression is achieved, it is recommended to 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, it is recommended to evaluate column efficiency using your standard operating procedures or the procedure described in Section 5.

## Section 5: Column Packing Evaluation

When column packing is complete, equilibrate the column with up to 5 CV equilibration buffer. 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. Minimum theoretical plate values should be 1,000–3,000 plates/m for linear velocities of 50–500 cm/hr.

$$\text{HETP} = L/N$$

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

L = Bed height (cm)

N = Number of theoretical plates

$V_e$  = 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

Peaks should be symmetrical and the asymmetry factor as close as possible to 1. Values of 0.8–1.8 are acceptable.

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  $V_e$

$A_s = 0.8\text{--}1.8$  is acceptable.

## Section 6: Operation and Maintenance

Nuvia™ media are designed to achieve the highest productivity (grams of drug per operational hour per liter of media) possible. Nuvia media should be run at the highest linear velocities and loading capacities allowed by the column and the chromatography system. A linear flow rate of 300 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 elution buffer, modifying the gradient profile, or experimenting with different buffer salts.

Note: Due to the high binding capacity of Nuvia media, it is recommended to elute the biomolecule at flow rates <150 cm/hr to minimize viscosity induced backpressure.

Figure 1 shows the effect of flow rate on backpressure.

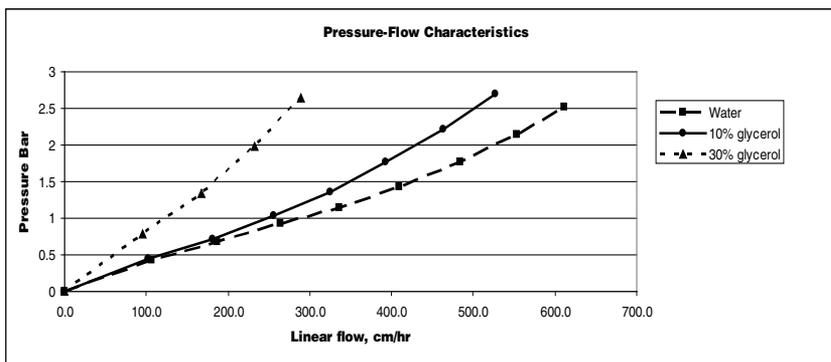


Fig. 1. Nuvia S media pressure/flow performance for a 20 cm diameter x 20 cm bed height column packed to compression factor of 1.17.

Figure 2 shows the effect of conductivity on Nuvia S media binding capacity.

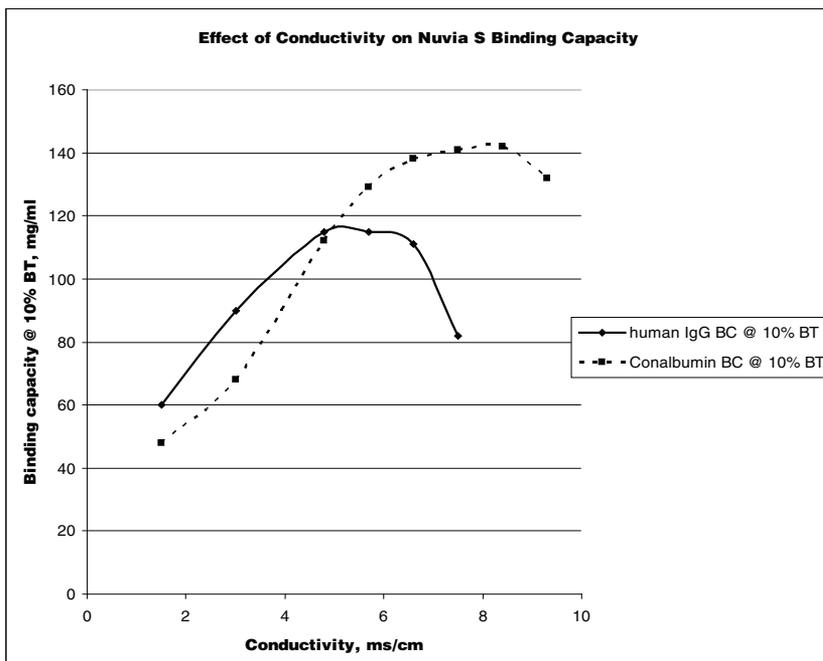


Fig 2. Effect of conductivity on Nuvia S media binding capacity at pH 5.0. Column: 1.1 x 9.3 cm; flow rate: 9.75 ml/min (600 cm/hr); sample: 5 mg/ml human IgG or 5 mg/ml conalbumin.

Figure 3 shows how the effect of conductivity on Nuvia Q media binding capacity.

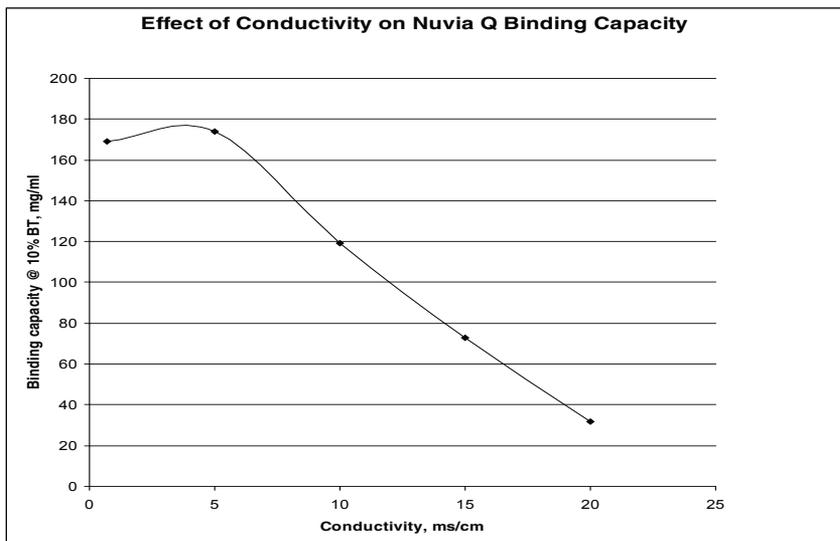


Figure 4 shows how binding capacity for human IgG can be achieved at higher conductivity and lower load buffer pH.

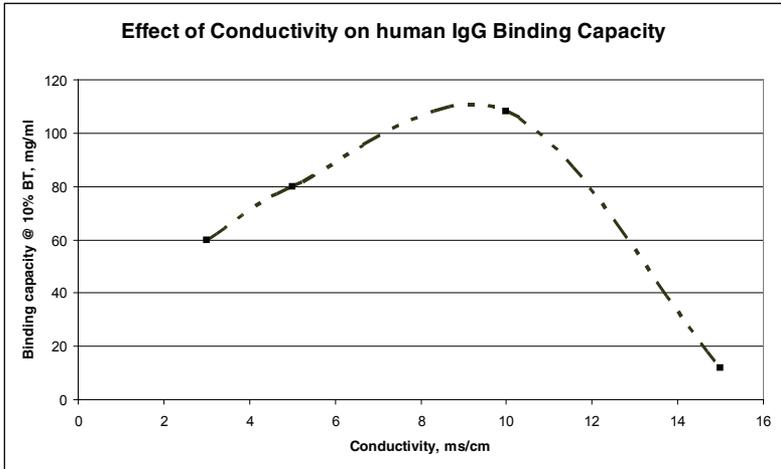


Fig. 4. Effect of conductivity on polyclonal h.IgG binding capacity of Nuvia S media at pH 4.5. Column: 0.7 x 5.5 cm; flow rate: 2.0 ml/min (300 cm/hr); sample: 4.5 mg/ml human IgG in 20 mM sodium acetate, pH 4.5.

All buffers commonly used for ion exchange chromatography can be used with Nuvia media (see 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. Common buffers for ion exchange chromatography.**

<b>Buffer</b>	<b>Buffering Range</b>
<b>Nuvia S</b>	
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
<b>Nuvia Q</b>	
Bicine	7.6–9.0
Bis-Tris	5.8–7.2
Diethanolamine	8.4–8.8
Diethylamine	9.5–11.5
L-histidine	5.5–6.0
Imidazole	6.6–7.1
Pyridine	4.9–5.6
Tricine	7.4–8.8
Triethanolamine	7.3–8.3
Tris	7.5–8.0

## Section 7: Regeneration and Sanitation

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 40 min is recommended.

## Section 8: Cleaning-in-Place (CIP) and Sanitation

If a column no longer yields reproducible results, the media may require thorough CIP and sanitation 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, and 6 M guanidine hydrochloride.

## Section 9: Storage

For long-term storage, Nuvia™ 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™ 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
156-0311	Nuvia™ S Media, 25 ml
156-0313	Nuvia S Media, 100 ml
156-0315	Nuvia S Media, 500 ml
156-0317	Nuvia S Media, 10 L
156-0411	Nuvia Q Media, 25 ml
156-0413	Nuvia Q Media, 100 ml
156-0415	Nuvia Q Media, 500 ml
156-0417	Nuvia Q Media, 10 L

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

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