
Nuvia cPrime Hydrophobic Cation Exchange Media

Instruction Manual

Catalog numbers

1563401

1563402

156-3403

156-3404

156-3405

156-3406

Please read the instructions in this manual prior to using Nuvia cPrime Hydrophobic Cation Exchange Media. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories, Inc. representative.

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Section 1

Introduction

Nuvia cPrime Hydrophobic Cation Exchange Media are designed for the process-scale purification of a wide variety of therapeutic proteins. Nuvia cPrime Media's unique selectivity allows method developers to use hydrophobic and cation exchange interaction modes to achieve effective purification. More importantly, the media have a wide design space for binding and elution, allowing for the development of highly robust methods in a commercial manufacturing setting. Nuvia cPrime Media are built on a rigid, mechanically and chemically stable macroporous base matrix with a particle size optimized to deliver exceptional flow properties, fast mass transfer, and stability. See the Nuvia cPrime Media product information sheet ([bulletin 6242](#)) for more product details.

If you have questions or require method development assistance with Nuvia cPrime Media, contact your local Bio-Rad process chromatography representative or the Bio-Rad chromatography technical support group for assistance online at [bio-rad.com/processhelp](https://www.bio-rad.com/processhelp), by email at process@bio-rad.com, or by phone (see last page/back cover for local phone numbers).

Section 2

Technical Description

Table 1. Nuvia cPrime Media technical description.

Functional group	Hydrophobic cation exchanger
Base matrix composition	Macroporous highly crosslinked hydrophilic polymer
Particle size	70 $\mu\text{m} \pm 10 \mu\text{m}$
Dynamic binding capacity*	$\geq 40 \text{ mg/ml}$
Ligand density	103–143 $\mu\text{eq/ml}$
Recommended linear flow rate range	50–600 cm/hr
Pressure vs. flow performance**	Under 2 bar at a flow rate of 600 cm/hr
pH stability	2–14 short term 3–13 long term
Chemical stability	1.0 N NaOH, 1.0 N HCl, 25% HOAc, 8 M urea, 6 M Gu-HCl, 6 M KSCN, 3 M NaCl, 1% Triton X-100, 2% SDS + 0.25 M NaCl, 20% ethanol, 70% ethanol, 30% isopropanol
Shipping solution	20% ethanol
Storage conditions	20% ethanol
Shelf life***	5 years

* At 10% breakthrough hlgG.

** 20 x 20 cm packed bed (1.17 compression factor).

***Stored at room temperature in 20% ethanol under accelerated conditions.

Section 3

Preparation

Nuvia cPrime Media are supplied fully hydrated in 20% ethanol as a 50% (v/v) slurry. For column packing, removal of the shipping buffer is recommended. Small volumes of Nuvia cPrime Media are easily washed in a Büchner funnel with 4–5 volumes of packing buffer. For large volume preparation, cycle through 3–4 settling and decanting steps using the column packing buffer in the shipping container.

Removal of fines from Nuvia cPrime Media is not required. If, however, particle fines have been generated during handling, resuspend the settled media and remove any opaque or cloudy supernatant before resettling is complete. Repeat several times until supernatant is clear.

Section 4

Column Packing

Nuvia cPrime Media 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 cPrime Media in a conventional laboratory-scale column with an internal diameter of 5–50 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, 20–50 mM buffer salt (see Table 2) 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, prepare an aliquot of Nuvia cPrime Media in a graduated cylinder to determine the slurry percentage.
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 impeller at low to moderate speed.
5. Using a compression factor of 1.17, calculate the volume of slurry required for the intended bed height.

For example, for a 20 cm bed height using a 50% slurry, the volume would be:

$$1.17 (20/0.5) * \pi r^2$$

6. Add a small amount of packing buffer to the column to wet the bottom frit, then pour in calculated amount of resin slurry.
7. 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 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

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 cPrime Media slurry through the packing pump. Use a low-shear impeller for automatic mixing or a plastic paddle for manual mixing to avoid damaging the media. The best overall performance of Nuvia cPrime Media will be obtained with a compression ratio of 1.15–1.20. Compression factor is defined as settled bed height divided by packed bed height.

After achieving the desired compression ratio, it is recommended to condition the column by flowing fresh packing or equilibration buffer for 3 CV 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.

Flow Properties

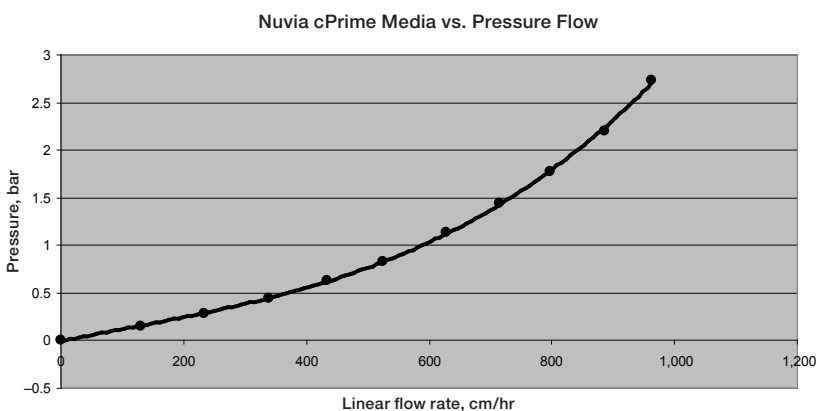


Fig. 1. Nuvia cPrime Media pressure vs. flow performance for a 20 cm diameter column and a 20 cm bed height; compression ratio 1.17. Nuvia cPrime Media have fast mass transfer properties, enabling users to achieve high productivity at fast flow rates. The media should be run at the highest linear velocities that facilitate good separation and are allowed by the column and chromatography system specifications. A linear flow rate of 300 cm/hr and a 20 cm bed height is recommended as a starting point.

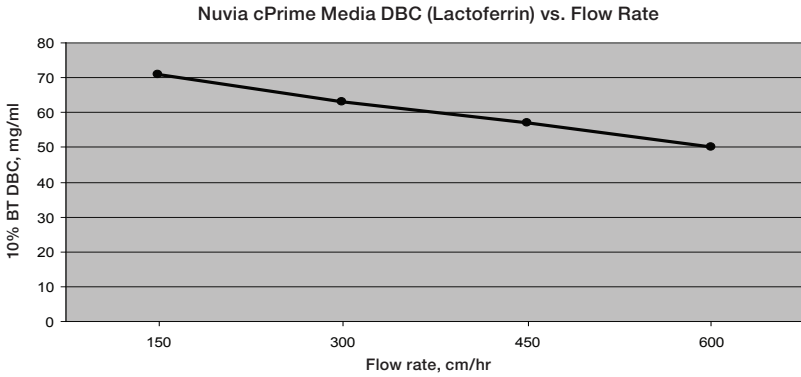


Fig. 2. Effect of flow rate on Nuvia cPrime Media binding capacity for lactoferrin.

Column dimension: 1.1 x 9.6 cm

Sample: 5.25 mg/ml lactoferrin

Buffers

All buffers commonly used for ion exchange chromatography can be used with Nuvia cPrime Media.

Table 2. Common buffers for cation exchange chromatography. A buffer concentration of 60 mM is recommended for most buffers.

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
Phosphate	6.5–8.0
Tris	7.5–9.0

Section 5

Column Packing Evaluation

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 height equivalent to a theoretical plate (HETP). 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 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

$A_s = 0.8$ – 1.8 is acceptable

Section 6

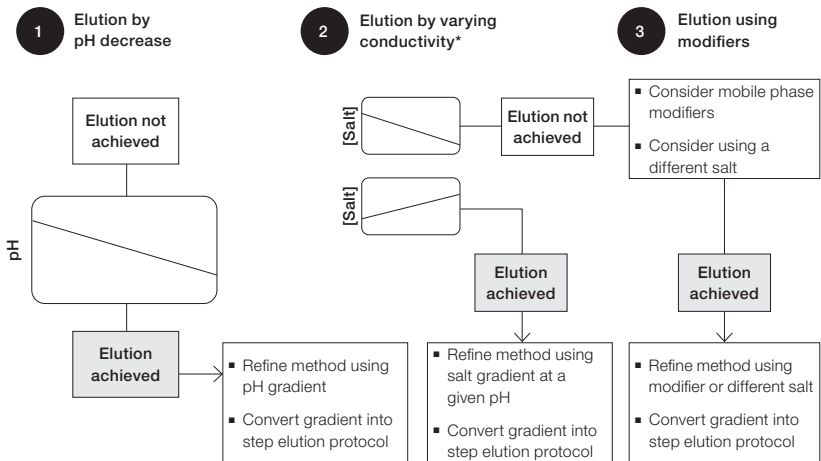
Method Development

Developing an effective and robust method with Nuvia cPrime Media is straightforward. Below 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 cPrime Media are determined chiefly by pH and salt. The high salt tolerance of the media often allows for direct loading at high conductivity. An increase in pH will, in most cases, achieve elution. Conductivity is another way to achieve and/or optimize elution, and the final method is often a combination of an increase 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 DOE studies 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 without dilution onto the Nuvia cPrime Media column. To elute, use an increasing pH gradient. If satisfactory elution and recovery are achieved, refine and/or make a step gradient to complete the step (range pH 4–8, depending on protein).
2. If elution is not satisfactory after step 1, run a salt gradient to disrupt electrostatic or hydrophobic interactions that may be preventing elution or broadening the peak. Use the pH from step 1 that achieved the best elution. The 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 your local Bio-Rad process chromatography representative or the Bio-Rad chromatography technical support group for assistance online at bio-rad.com/processhelp, by email at process@bio-rad.com, or by phone (see last page/back cover for local phone numbers).

Section 7

Sanitization and Regeneration

After each chromatography run, the packed media 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 0.1 N NaOH to remove remaining proteinaceous impurities. Washing should be conducted until absorbance returns to baseline. The column is now ready to be sanitized in 1.0 N NaOH.

After sanitization, to equilibrate the column we recommend applying 4–6 CV of a solution such as 60 mM sodium acetate.

Note: if the column no longer yields reproducible results, the media may require additional cleaning to remove strongly bound contaminants. Acceptable cleaning agents include 25% acetic acid, 8 M urea, 1% Triton X-100, 6 M potassium thiocyanate, 70% ethanol, 30% isopropyl alcohol, 1 N NaOH, or 6 M guanidine hydrochloride.

Section 8

Storage

For long-term storage, Nuvia cPrime Media should be stored with 0.1 N NaOH or 20% ethanol.

Section 9

Regulatory Support

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

Section 10

Ordering Information

Catalog #	Description
1563401	Nuvia cPrime Media , 25 ml
1563402	Nuvia cPrime Media , 100 ml
156-3403	Nuvia cPrime Media , 500 ml
156-3404	Nuvia cPrime Media , 1 L
156-3405	Nuvia cPrime Media , 5 L
156-3406	Nuvia cPrime Media , 10 L

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

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