EconoFit Nuvia aPrime 4A and EconoFit Nuvia cPrime Columns, 1 ml

Instruction Manual

Catalog numbers

12009280
12009281

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Section 1
Introduction

EconoFit Nuvia aPrime 4A and Nuvia cPrime Columns are convenient, disposable, prepacked low-pressure chromatography columns. They facilitate both increased run-to-run uniformity and high purity of proteins through the column design and novel resin technology. Compatible with most aqueous buffers commonly used for protein purification, EconoFit Columns offer improved performance for your protein separation needs.

These columns are packed with Nuvia aPrime 4A or Nuvia cPrime Mixed-Mode Resins from Bio-Rad. Nuvia beads are produced using controlled polymerization of water-soluble acrylamido and vinylic monomers and exhibit low nonspecific binding due to the hydrophilic nature of the polymers. Nuvia aPrime 4A Resin is a hydrophobic anion exchange resin and Nuvia cPrime Resin is a hydrophobic cation exchange resin. Both can function effectively across a wide range of salt concentrations and pH, making them suitable for easy integration into a multistep process. The resins' ligand density and hydrophobicity are designed to facilitate selective and readily reversible binding of target molecules for maximal purity and recovery. The ligand design permits straightforward purification method development. These resins can be used to purify both established therapeutic proteins and diverse new constructs in development, including those that lack affinity handles. They are also effective in the purification of salt- and pH-sensitive proteins with a high propensity for aggregation and/or degradation.

Section 2
Product Information

EconoFit Columns are disposable, easy-to-use, prepacked chromatographic columns that are supplied ready for use in convenient 1 and 5 ml sizes. They can be quickly connected to liquid chromatography systems using 10-32 fittings. Columns are available for a variety of chromatographic techniques, including desalting (size exclusion [SEC]), ion exchange (IEX), affinity (AC), mixed-mode, and hydrophobic interaction chromatography (HIC). Refer to **bio-rad.com/ResinsandColumns** for a complete listing of products in the EconoFit Column portfolio.

See Table 1 for the EconoFit Nuvia aPrime 4A and cPrime Column information and Table 2 for the columns' technical specifications.

Table 1. EconoFit Nuvia aPrime 4A and cPrime Column specifications.

Property	Description
Size	1 ml bed volume
Bed dimension	25 mm length x 7 mm inner diameter
Fittings	10-32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Autoclavability	Not autoclavable

Table 2. Nuvia aPrime 4A and cPrime Resin specifications.

Property	Nuvia aPrime 4A	Nuvia cPrime
Type of ion exchanger	Hydrophobic anion exchanger	Hydrophobic cation exchanger
Functional group	Aromatic hydrophobic anion exchanger	Aromatic hydrophobic weak cation exchanger
Particle size range	50 ± 10 µm	70 ± 10 µm
Ligand density	100 ± 20 µeq	125 ± 25 µeq
Dynamic binding capacity	≥50 mg/ml at 300 cm/hr*	>40 mg/ml hIgG at 300 cm/hr*** >60 mg/ml lactoferrin
Recommended linear flow rate	50–300 cm/hr	50–600 cm/hr
pH stability	2–14 short term**	3–14 short term 4–13 long term
Shipping solution	20% ethanol + 1.0 M NaCl	20% ethanol
Regeneration	1.0 M NaOH	1–2 M NaCl
Sanitization	1.0 M NaOH	1.0 M NaOH
Storage conditions	20% ethanol	20% ethanol
Storage temperature	RT	RT
Chemical stability	1 M NaOH, 1 M HCl, 25% acetic acid, 8 M urea, 6 M guanidine HCl, 3 M NaCl, 1% Triton X-100, 20–70% ethanol, 30% IPA	1 M NaOH, 1 M HCl, 25% acetic acid, 8 M urea, 6 M guanidine HCl, 3 M NaCl, 1% Triton X-100, 20–70% ethanol, 30% IPA, 6 M KSCN, 2% SDS + 0.25 NaCl
Shelf life	5 years	5 years

* 10% breakthrough capacity determined with 1.0 mg/ml of an acidic monoclonal antibody (pI ~6.9) in 20 mM $NaPO_4$, pH 7.8.
** No significant change in ligand density after 200 hr contact at 22°C.
*** At 10% breakthrough.

Nuvia aPrime 4A and cPrime are also available in larger size bottles. Please refer to the ordering section for more information.

Section 3
Buffers and Methods

Developing an effective and robust method with Nuvia aPrime 4A or cPrime Resins is straightforward. The binding and elution mechanisms of these resins are affected chiefly by buffer pH and salt. Their salt tolerance often allows for direct loading at high conductivity. A decrease in pH will in many cases achieve elution. Changes in ionic strength can also achieve and/or optimize elution and the final method is often a combination of a decrease 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.

In most cases, conducting a few simple design of experiments (DOE) to identify optimal binding and elution conditions will yield an effective, robust, and scalable method. Detailed method development guidelines and rationale can be found in Bio-Rad bulletins 10000098359 and 10023853. For best results, and increased column life, samples and buffers should be degassed and filtered through a 0.45 µm filter.

All buffers commonly used for anion and cation exchange chromatography as listed in Table 3 can be used with Nuvia aPrime 4A and cPrime, respectively.

Table 3. Common buffers for ion exchange chromatography.

Type of Buffering	Ion Exchange Buffer Range, pH
Anion	
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	7.8–8.9
Triethanolamine	7.3–8.0
Tris	7.5–8.0
Cation	
Acetic acid	4.8–5.2
Citric acid	4.2–5.2
HEPES	7.6–8.2
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
Pivalic acid	4.7–5.4
TES	7.2–7.8
Tricine	7.8–8.9

Section 4
Preparing a Column and Subsequent Purification

EconoFit Nuvia aPrime 4A and cPrime Columns contain the fully hydrated 50% (v/v) slurry in 20% ethanol + 1 M NaCl as the storage solution. These supports are ready to use after equilibrating the column in the buffer of choice. To perform a buffer exchange, connect the column to a liquid chromatography system and condition it as instructed below:

1. Set pump flow rate to 3.0 ml/min (731 cm/hr).
2. Wash the column with degassed low-salt buffer for 2 min.
3. Wash the column with degassed high-salt buffer for 5 min.
4. Equilibrate the column with low-salt buffer for 5 min.
5. Reduce the flow rate to the rate that will be used in the purification protocol.

Sample Preparation

Proper pH and ionic strength are necessary for consistent and reproducible results. Sample can be exchanged into the starting buffer or diluted to the starting buffer concentration. This can be achieved by diluting the sample to the ionic strength of the starting buffer, dialyzing against the starting buffer, or exchanging it into the starting buffer. Buffer exchange can be accomplished using the EconoFit Bio-Gel P-6 Desalting Column; Bio-Gel P-6DG, Micro Bio-Spin P-6, Micro Bio-Spin P-30, Bio-Spin P-6, or

Bio-Spin P-30 Columns; or Econo-Pac 10DG Desalting Columns, as listed in Table 4. The choice of product will depend on the sample volume. All samples should be filtered through a 0.45 µm filter prior to column application.

Table 4. Product for buffer exchange.

Sample Volume	Recommended Product	Use	Catalog #
10–75 µl	Micro Bio-Spin P-6 Column	Desalting proteins over 6 kD	7326221
10–75 µl	Micro Bio-Spin P-30 Column	Desalting proteins over 30 kD	7326223
50–100 µl	Bio-Spin P-6 Column	Desalting proteins over 6 kD	7326227
50–100 µl	Bio-Spin P-30 Column	Desalting proteins over 30 kD	7326231
100 µl–3 ml	EconoFit Bio-Gel P-6 Desalting Column	Desalting proteins over 6 kD	12009239
Up to 3 ml	Econo-Pac 10DG Desalting Columns	Desalting proteins over 6 kD	7322010
Unlimited	Bio-Gel P-6DG Gel	Desalting proteins over 6 kD	1500738

Section 5
Scaling Up

The EconoFit Nuvia aPrime 4A and cPrime Columns are available in a 1 ml format. The Nuvia aPrime 4A and cPrime Resins are also available in various amounts, from small bottles to larger bulk quantities, for scaling up methods developed using the columns. For quick scale-up, two or three columns of the same type can be connected in series, so take care to maintain an overall system pressure ≤45 psi.

In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory to process scale. Ask your local Bio-Rad representative or go to **bio-rad.com/ResinsandColumns** for more information.

Section 6
Regenerating, Cleaning, Sanitizing, and Storing Columns

Protein cross-contamination, frit clogging, and increased backpressure can result from running a column beyond the recommended number of uses. After repeated use, a column may run slower or produce high backpressure. We recommend that you dispose of a column after several uses. To avoid cross-contamination, designate each column for a single protein. To maintain good flow properties, clean the columns between uses. Acceptable clean in place (CIP) agents include 25% acetic acid, 8 M urea, 1% Triton X-100, 70% ethanol, 30% isopropyl alcohol, and 6 M guanidine hydrochloride. Run the cleaning protocol at 2 ml/min. The following cleaning and regeneration procedure may be used:

1. Sanitize the support in the column with 2–4 bed volumes of 1.0 M NaOH at 50–100 cm/hr while maintaining a minimum contact time of 40 min.

2. To reequilibrate the columns, wash the Nuvia aPrime 4A Column with 2–4 bed volumes of 1 M NaOH or the Nuvia cPrime Column with 0.5–2 M NaCl solution containing 50–100 mM buffer.

3. If lipid removal is required, the column may be washed with a 20–50% ethanol solution at 50 cm/hr.

Storage

After washing the columns with deionized water, EconoFit Columns should be purged and stored with PBS containing 0.5% NaN_3 or in 20% (v/v) ethanol solution, and capped for extended storage.

Section 7
Troubleshooting Guide

Possible Causes	Possible Solutions
Column Clogging or Slow Flow Rate	
Particulates in sample	Filter all samples and buffers through 0.2 µm filter prior to application
No Target Protein in Eluate	
Low level of target	Check expression level of protein in starting SDS-PAGE material
Target is in flowthrough	Optimize binding conditions
Target is not eluted	Optimize elution buffer
Precipitation during Purification	
Binding capacity of column exceeded	Load less sample
Protein aggregating	▪ Include low amount of detergent (0.1% Triton X-100, Tween 20) ▪ Include glycerol up to 10% ▪ Optimize buffer pH and salt concentration

Section 8
Ordering Information

Catalog #	Description
EconoFit Nuvia aPrime 4A and cPrime Columns	
12009280	**EconoFit Nuvia aPrime 4A Column**, 1 x 1 ml column
12009281	**EconoFit Nuvia cPrime Column**, 1 x 1 ml column
Nuvia aPrime 4A and cPrime Resin Bottles and Plates	
12007397	**Nuvia aPrime 4A Resin**, 25 ml
12007396	**Nuvia aPrime 4A Resin**, 100 ml
12007379	**Nuvia aPrime 4A Resin**, 500 ml
12007380	**Nuvia aPrime 4A Resin**, 5 L
12007391	**Nuvia aPrime 4A Resin**, 10 L
12007411	**Foresight Nuvia aPrime 4A Plates**, 2 x 96-well, 20 µl
12007392	**Foresight Nuvia aPrime 4A Column**, 1 ml
12007393	**Foresight Nuvia aPrime 4A Column**, 5 ml
12007394	**Foresight Nuvia aPrime 4A RoboColumn Unit**, 200 µl
12007395	**Foresight Nuvia aPrime 4A RoboColumn Unit**, 600 µl
732-4705	**Foresight Nuvia cPrime Plates, 2 x 96-well**, 20 µl
732-4807	**Foresight Nuvia cPrime RoboColumn Unit**, 200 µl
732-4808	**Foresight Nuvia cPrime RoboColumn Unit**, 600 µl
732-4722	**Foresight Nuvia cPrime Column**, 1 ml
732-4742	**Foresight Nuvia cPrime Column**, 5 ml
1563401	**Nuvia cPrime Resin**, 25 ml
1563402	**Nuvia cPrime Resin**, 100 ml
156-3403	**Nuvia cPrime Resin**, 500 ml
156-3404	**Nuvia cPrime Resin**, 1 L
156-3405	**Nuvia cPrime Resin**, 5 L
156-3406	**Nuvia cPrime Resin**, 10 L

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

Section 9
Bibliography

Drevland et al. Improved Process Economics of HUMIRA Biosimilar Purification with Ion Exchange and Mixed-Mode Resin. Bio-Rad Bulletin #7130.

Fitchmun et al. Development of an Efficient Manufacturing Process for Adenovirus. Bio-Rad Bulletin #6719.

Harris ELV and Angal S (1989). Protein Purification Methods: A Practical Approach (Oxford, UK: IRL Press).

He et al. Purification of Recombinant Proteins on Nuvia cPrime Hydrophobic Cation Exchange Resin: A Simple Approach to Method Development. Bio-Rad Bulletin #6418.

He et al. Purification of Antibodies with Nuvia aPrime 4A Hydrophobic Anion Exchange Resins, Bio-Rad Bulletin #7193.

Loncar et al. Single-Step Purification of Raw Starch Digesting α-Amylase Using Nuvia cPrime Hydrophobic Cation Exchange Resin. Bio-Rad Bulletin #6992.

Loncar et al. Purification of a Recombinant Bacterial DyP-Type Peroxidase with a Hydrophobic Anion Exchange Resin. Bio-Rad Bulletin #7182.

Scopes RK (1987). Protein Purification: Principles and Practice (Second Edition) (New York, NY: Springer-Verlag).

Snyder LR and Kirkland JJ (1979). Introduction to Modern Liquid Chromatography (Second Edition) (New York, NY: Wiley).

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