EconoFit Nuvia HR-S Columns, 1 ml

Instruction Manual

Catalog number

12009284

Please read the instructions in this manual prior to using EconoFit Nuvia HR-S Columns. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Table of Contents

Section 1 Introduction 1

Section 2 Product Information. 1

Section 3 Buffers and Methods. 2

Section 4 Preparing a Column and Subsequent Purification 3

Section 5 Scaling Up. 3

Section 6 Regenerating, Cleaning, Sanitizing, and Storing Columns. 4

Section 7 Troubleshooting Guide 5

Section 8 Ordering Information 6

Section 9 Bibliography 6

Section 1
Introduction

EconoFit Nuvia HR-S 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 aqueous buffers most commonly used for protein purification, EconoFit Columns offer improved performance for your protein separation needs.

These columns are packed with Bio-Rad's Nuvia HR-S Cation Exchange Resin. It is built on the rugged UNOsphere Base Bead, which provides fast mass transfer kinetics, excellent flow characteristics, and robust chemical stability against common caustic cleaning protocols. The particle size and chemistry of Nuvia HR-S offers exceptional resolution and high recovery, thereby delivering excellent process productivity and economics. Nuvia HR-S Resin can be used for intermediate and polish purifications where separation of closely related molecules is required.

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 HR-S Column information and technical specifications.

Table 1. EconoFit Nuvia HR-S Column specifications.

Property	Description
Size	1 ml bed volume
Bed dimensions	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
Type of ion-exchanger	Strong cation
Functional group	$-SO_3^-$
Particle size range	50 ± 10 µm
Total ionic capacity	100–180 µeq
Dynamic binding capacity*	>70 mg/ml at 300 cm/hr
Recommended linear flow rate	50–200 cm/hr
pH stability	2–14 short term 4–13 long term**

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Property	Description
Shipping solution	20% ethanol + 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 M NaOH
Storage conditions	20% ethanol or 0.1 M NaOH
Storage temperature	RT
Chemical stability	1.0 M NaOH (20ºC) for up to 5 weeks (840 hr) 0.1 M NaOH (20ºC) for up to 5 years
Shelf life	5 years
Autoclavability	Not autoclavable

* 10% breakthrough capacity determined with 5.0 mg/ml human IgG in 20 mM Na acetate, pH 5.0.

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

Nuvia HR-S is also available in larger sizes in bottles. Please refer to the ordering section for more information.

Section 3
Buffers and Methods

Ion exchange chromatography is usually performed using increasing salt gradients or pH gradients to elute the sample components. For best results, and increased column life, samples and buffers should be degassed and filtered through a 0.45 µm filter.

Common buffers for cation exchange chromatography are listed in Table 2.

An appropriate starting point for purifying samples is a linear gradient from 0–0.4 M NaCl spanning 1–20 column volumes at 120 cm/hr for the 1 ml column. The separation can be optimized by changing the gradient profile. At the end of each run the column can be regenerated with 1–2 M NaCl followed by equilibration buffer. Return to the desired flow rate and proceed with the next separation. For further regeneration methods, refer to section 6.

Table 2. Buffers compatible with Nuvia HR-S Resin.

Buffer	Buffering Range, pH
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 4
Preparing a Column and Subsequent Purification

EconoFit Nuvia HR-S Columns contain the fully hydrated 50% (v/v) slurry in 20% ethanol + 0.1 M NaCl as the storage solution. This support is 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:

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 EconoFit Bio-Gel P6 Desalting Columns, Micro Bio-Spin P-6 or Micro Bio-Spin P-30 Columns, Bio-Spin P-6 or Bio-Spin P-30 Columns, Econo-Pac 10DG Desalting Columns, or Bio-Gel P-6DG Gel, as listed in Table 3. 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 3. 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 P6 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

EconoFit Nuvia HR-S Columns are available in a 1 ml format. Nuvia HR-S Resin is also available in various amounts, from 25 ml 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, 6 M potassium thiocyanate, 70% ethanol, 30% isopropyl alcohol, 1.0 M HCl, 1.0 M NaOH, 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 regenerate the column, wash the column with 2–4 bed volumes of 0.5–2.0 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 Ion Exchange 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 not bound	Change the equilibration buffer
Target is in flowthrough	Optimize binding conditions
Target is not eluted	Recheck and optimize the elution buffer and conditions
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 HR-S Columns	
12009284	**EconoFit Nuvia HR-S Column**, 1 x 1 ml column
Nuvia HR-S Resin Bottles and Plates	
1560511	**Nuvia HR-S Media**, 25 ml
1560513	**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** Plate, 20 µl
732-4831	**Foresight Nuvia HR-S RoboColumn Unit**, 200 µl
732-4832	**Foresight Nuvia HR-S RoboColumn Unit**, 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.

Section 9
Bibliography

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