EconoFit Macro-Prep High Q-3HT Columns, 1 ml

Instruction Manual

Catalog #12009283

Please read the instructions in this manual prior to using EconoFit Macro-Prep High Q-3HT Columns. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Section 1
Introduction

EconoFit Macro-Prep High Q-3HT Columns are convenient, disposable, prepacked low-pressure chromatography columns. EconoFit Columns offer 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 Bio-Rad's specially designed Macro-Prep High Q-3HT Resin. Macro-Prep High Q-3HT Resin is a strong anion exchanger containing quaternary amine functional groups, ideal for purifying acidic and neutral proteins and peptides. This resin is an excellent choice for rapid purification. The Macro-Prep methacrylate copolymer bead provides high-resolution separations at high flow rates. Macro-Prep is a polymer matrix that contains carboxyl groups in the backbone. Depending on the application, pH conditions, and samples, these charges can contribute to the mode of interaction. This property can be exploited to give unique selectivity, which could increase yield and purity.

Section 2
Product Information

EconoFit Columns are disposable, easy-to-use, prepacked chromatographic columns, which 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), ion exchange, affinity, mixed-mode, and hydrophobic interaction chromatography. See Table 1 for column information and technical specifications. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of products in the EconoFit Column portfolio.

Table 1. EconoFit Macro-Prep High Q-3HT Column information and specifications.

Property	Description
Size	1 ml bed volume
Bed dimension	7 mm inner diameter x 25 mm length
Fittings	10-32 (1/16"), female inlet and male outlet
Maximum pressure tolerance (column hardware)	72 psi/5 bar/0.5 MPa
Column material	Polypropylene
Frit material	High-density polyethylene
Type of ion exchanger	Strong anion
Functional group	$-N^{+}(CH_3)_3$
Mean particle size	50 µm
Dynamic binding capacity	≥37 mg bovine serum albumin/ml
Recommended linear flow rate	50–600 cm/hr
pH stability	1–10*
Shipping solution	20% ethanol + 0.1 M NaCl
Regeneration	2–4 column volumes (CV) of a high salt buffer (0.5–2.0 M)
Sanitization	3–5 CV of acetic acid or phosphoric acid solution, pH ≥1.5
Storage conditions	Acetic acid or phosphoric acid solution, pH ≥1.5
Storage temperature	Room temperature
Chemical stability	All commonly used neutral or acidic pH cleaning agents
Shelf life	5 years
Autoclavability	Not autoclavable

* The use of basic reagents with a pH >10 should be evaluated for each application.

Macro-Prep High Q-3HT Resin is also available in larger sizes in bottles. Refer to the ordering information 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 anion 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 CV 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 starting buffer. Return to the desired flow rate and proceed with the next separation.

Table 2. Buffers compatible with Macro-Prep High Q-3HT 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
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 4
Preparing a Column and Subsequent Purification

EconoFit Macro-Prep High Q-3HT Columns contain the fully hydrated 50% (v/v) slurry in 20% ethanol 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 follows:

1. Set the pump flow rate to 3.0 ml/min.
2. Wash the column with degassed high-salt buffer for 5 min.
3. Wash the column with degassed low-salt buffer for 2 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. The 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 Micro Bio-Spin P-6 or P-30 Gel Column, Bio-Spin P-6 or P-30 Gel Column, EconoFit Bio-Gel P-6 Desalting Column, Econo-Pac 10DG Desalting Column, 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. Products for buffer exchange.

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

Section 5
Scaling Up

The EconoFit Macro-Prep High Q-3HT Columns are available in a 1 ml format. Macro-Prep High Q-3HT 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 cleaning-in-place (CIP) agents include 25% acetic acid, 8 M urea, neutral or cationic detergents, 6 M potassium thiocyanate, 70% ethanol, 30% isopropyl alcohol, and 6 M guanidine HCl. 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 acetic acid or phosphoric acid solution, pH ≥1.5, while maintaining a minimum contact time of 40 min.
2. To re-equilibrate the column, wash the column with 2–4 bed volumes of 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 Ion Exchange Columns should be purged and stored in the recommended storage solutions provided in Table 1 and capped for extended storage.

Section 7
Troubleshooting Guide

Problem	Possible Cause	Solution
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
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% ▪ Check sample stability in buffers
Loss of binding capacity after cleaning with SDS	SDS binds tightly	Wash the column with 3–5 CV of 30% isopropanol + 1 M NaCl

Section 8
Ordering Information

Catalog #	Description
12009283	**EconoFit Macro-Prep High Q-3HT Column**, 1 x 1 ml column
1560043-3HT	**Macro-Prep High Q-3HT Resin**, 10 L bottle

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

Section 9
Bibliography

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