EconoFit Macro-Prep High Q, High S, DEAE, and CM Columns, 1 and 5 ml

Instruction Manual

Catalog numbers

12009275
12009267
12009268
12009269
12009276
12009270
12009271
12009272
12009274
12009264
12009265
12009266
12009273

Please read the instructions in this manual prior to using EconoFit Macro-Prep High Q, High S, DEAE, and CM 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

EconoFit Macro-Prep High Q, High S, DEAE, and CM Columns are convenient, disposable, prepacked low-pressure chromatography columns. EconoFit Columns offer both increased run-to-run uniformity and high purity of protein 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.

EconoFit Macro-Prep High Q, High S, DEAE, and CM Columns are packed with Macro-Prep Ion Exchange Media. These media are based on hydrophilic, spherical, polymeric beads designed for the purification of proteins, nucleic acids, viruses, plasmids, and other macromolecules. Macro-Prep Beads are designed to provide medium capacity, low backpressure, and high productivity.

Section 2
Product Information

EconoFit Columns are disposable, easy-to-use, prepacked chromatographic columns supplied ready for use in convenient 1 and 5 ml sizes. They are 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). See Table 1 for specifications. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of items in the EconoFit Column product line.

Table 1. EconoFit Macro-Prep High Q, High S, DEAE, and CM Column specifications.

Property	Description
Size	1 and 5 ml bed volumes
Bed dimensions	1 ml: 25 mm length x 7 mm inner diameter 5 ml: 25 mm length x 16 mm inner diameter
Operational flow rates	1 ml: 0.5–6 ml/min (78–935 cm/hr) 5 ml: 2.5–15 ml/min (75–448 cm/hr)
Maximum operating pressure	72 psi
Fittings	10-32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Shipping solution	20% ethanol
Storage conditions	20% ethanol
Autoclavability	Not autoclavable

Macro-Prep High Q, High S, DEAE, and CM Resins are also available in bottles. Refer to Ordering Information in section 8 of this manual. See Table 2 for specifications. Go to **bio-rad.com/ResinsandColumns** for more information.

Table 2. Macro-Prep High Q, High S, DEAE, and CM Resin specifications.

Property	High Q	High S	DEAE	CM
Type of ion exchanger	Strong anion	Strong cation	Weak anion	Weak cation
Functional group	$-N^+(CH_3)_3$	$-SO_3^-$	$-N^+(C_2H_5)_2$	$-COO^-$
Mean particle size*	50 µm	50 µm	50 µm	50 µm
Nominal pore size	1,000 Å	1,000 Å	1,000 Å	1,000 Å
Total ionic capacity	400 ± 75 µeq/ml	160 ± 40 µeq/ml	175 ± 75 µeq/ml	210 ± 40 µeq/ml
Dynamic binding capacity**	≥37 mg BSA/ml	≥49 mg IgG/ml	≥30 mg BSA/ml	≥25 mg hemoglobin/ml
Shipping counterion	Cl^-	Na^+	Cl^-	Na^+
Chemical stability				
1% SDS, 24 hr	Yes	Yes	Yes	Yes
6 M guanidine-HCl, 24 hr	Yes	Yes	Yes	Yes
pH stability	1–10	1–12	1–10	1–12
Antimicrobial agent	20% ethanol	20% ethanol	20% ethanol	20% ethanol
Regeneration	1–2 M NaCl	1–2 M NaCl	1–2 M NaCl	1–2 M NaCl
Storage conditions	20% ethanol	20% ethanol	20% ethanol	20% ethanol

* Mean particle size measured before derivatization.
** 10% breakthrough capacity determined in a 1.1 x 20 cm column.

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 and anion exchange chromatography are listed in Table 3.

An appropriate starting point for purifying samples is a linear gradient from 0–0.4 M NaCl spanning 1–20 column volumes at 0.5 ml/min for the 1 ml column, and 2.5 ml/min for the 5 ml column. The separation can be optimized by changing the gradient profile. At the end of each run the column can be regenerated with 1.0 M NaCl followed by starting buffer. Return to the desired flow rate and proceed with the next separation.

Table 3. Common buffers for ion exchange chromatography.

Type of Buffering	
Cation	**Ion Exchange Buffer Range, pH**
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
Anion	**Ion Exchange Buffer 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	7.8–8.9
Triethanolamine	7.3–8.0
Tris	7.5–8.0

Section 4
Preparing a Column and Subsequent Purification

EconoFit Macro-Prep High Q, High S, DEAE, and CM Columns contain 20% ethanol (v/v) as the storage solution. The fully hydrated support is ready to use after equilibrating the column in the buffer of choice. To perform buffer exchange, connect the column to a liquid chromatography system or peristaltic pump and condition it as instructed below:

1. Set pump flow rate to 3 ml/min for the 1 ml column or 6 ml/min for the 5 ml column.
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 P-6 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 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

EconoFit Columns are available in 1 and 5 ml formats. Macro-Prep High Q, High S, DEAE, and CM Resins are 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 ≤72 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

Regeneration

After each use, the column should be regenerated with the appropriate salt, in most cases 1–2 M NaCl in the presence of buffer. Wash with 2–4 column volumes of the buffered high salt solution. This reduces the potential for protein precipitation when selecting acid as a cleaning agent.

Cleaning

After repeated use, an ion exchange column may require thorough cleaning and regeneration to remove bound contaminants. Acceptable cleaning-in-place (CIP) reagents include 1% acetic acid/1% phosphoric acid with 0.4 M NaCl, acetic acid (up to 30%), 1% Triton X-100, ethanol (up to 70%), isopropyl alcohol (up to 30%), 8 M urea, and 6 M guanidine-HCl. Any of these agents can be combined in an appropriate cleaning protocol. As a general guide, we recommend the following.

1. Use high salt buffer for regeneration, as above.
2. For aggregated or precipitated proteins, or when dirty feedstock (such as crude lysate) has been used, wash with 3–5 column volumes of 6 M guanidine-HCl or 8 M urea at 0.75 ml/min for the 1 ml column and 3.5 ml/min for the 5 ml column.
3. For lipids or hydrophobically bound contaminants, wash with 0.1% Triton X-100 or 20–70% ethanol or isopropyl alcohol, or 1–30% acetic acid. Use 3–5 column volumes at 0.75 ml/min for the 1 ml column and 3.5 ml/min for the 5 ml column.
4. Remove additional contaminants with 0.4 M NaCl in 1% acetic acid/1% phosphoric acid. Use 3–5 column volumes at 0.75 ml/min for the 1 ml column and 3.5 ml/min for the 5 ml column.
5. If the column is to be used again immediately, wash with 2 column volumes of deionized water and 4–5 column volumes of starting buffer at 0.75 ml/min for the 1 ml column and 3.5 ml/min for the 5 ml column. Check the conductivity and pH of the effluent to verify that the column is equilibrated in the starting buffer before loading the sample.

Storage

After washing the columns with deionized water, EconoFit Ion Exchange Columns should be purged and stored with PBS containing 0.05% 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
Precipitation during Purification	
Binding capacity of column exceeded	Load less sample

Section 8
Ordering Information

Catalog #	Description
EconoFit Macro-Prep High Q Columns	
12009275	**EconoFit Macro-Prep High Q Column**, 1 x 1 ml column
12009267	**EconoFit Macro-Prep High Q Columns**, 5 x 1 ml columns
12009268	**EconoFit Macro-Prep High Q Column**, 1 x 5 ml column
12009269	**EconoFit Macro-Prep High Q Columns**, 5 x 5 ml columns
EconoFit Macro-Prep High S Columns	
12009276	**EconoFit Macro-Prep High S Column**, 1 x 1 ml column
12009270	**EconoFit Macro-Prep High S Columns**, 5 x 1 ml columns
12009271	**EconoFit Macro-Prep High S Column**, 1 x 5 ml column
12009272	**EconoFit Macro-Prep High S Columns**, 5 x 5 ml columns
EconoFit Macro-Prep DEAE Columns	
12009274	**EconoFit Macro-Prep DEAE Column**, 1 x 1 ml column
12009264	**EconoFit Macro-Prep DEAE Columns**, 5 x 1 ml columns
12009265	**EconoFit Macro-Prep DEAE Column**, 1 x 5 ml column
12009266	**EconoFit Macro-Prep DEAE Columns**, 5 x 5 ml columns
EconoFit Macro-Prep CM Columns	
12009273	**EconoFit Macro-Prep CM Column**, 1 x 1 ml column
Macro-Prep High Q Resins	
1580040	**Macro-Prep High Q Support**, 25 ml
1560040	**Macro-Prep High Q Support**, 100 ml
156-0041	**Macro-Prep High Q Support**, 500 ml
156-0042	**Macro-Prep High Q Support**, 5 L
156-0043	**Macro-Prep High Q Support**, 10 L
Macro-Prep High S Resins	
1580030	**Macro-Prep High S Support**, 25 ml
1560030	**Macro-Prep High S Support**, 100 ml
156-0031	**Macro-Prep High S Support**, 500 ml
156-0032	**Macro-Prep High S Support**, 5 L
156-0033	**Macro-Prep High S Support**, 10 L
Macro-Prep DEAE Resins	
1580020	**Macro-Prep DEAE Support**, 25 ml
1560020	**Macro-Prep DEAE Support**, 100 ml
156-0021	**Macro-Prep DEAE Support,** 500 ml
156-0022	**Macro-Prep DEAE Support**, 5 L
156-0023	**Macro-Prep DEAE Support**, 10 L
Macro-Prep CM Resins	
1580070	**Macro-Prep CM Support**, 25 ml
1560070	**Macro-Prep CM Support**, 100 ml
156-0071	**Macro-Prep CM Support**, 500 ml
156-0073	**Macro-Prep CM Support**, 10 L

Section 9
Bibliography

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