EconoFit UNOsphere Q and S Columns, 1 and 5 ml

Instruction Manual

Catalog numbers

12009301
12009302
12009303
12009304
12009305
12009306
12009307
12009308

Please read the instructions in this manual prior to using EconoFit UNOsphere Q and 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

EconoFit UNOsphere Q and S 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.

These columns are packed with Bio-Rad's UNOsphere Q and S Ion Exchange Supports. These supports are based on hydrophilic, spherical, polymeric beads designed for the purification of proteins, nucleic acids, viruses, plasmids, and other macromolecules. UNOsphere Beads provide high capacity, low backpressure, and high productivity.

Section 2
Product Information

EconoFit Columns are 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 UNOsphere Q and S 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
Maximum pressure tolerance	72 psi
Recommended flow rate	1 ml: 1–2 ml/min (240–480 cm/hr) 5 ml: 5–10 ml/min (240–480 cm/hr)
Maximum flow rate	1 ml: 4 ml/min (970 cm/hr) 5 ml: 20 ml/min (963 cm/hr)
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

UNOsphere Q and S 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. UNOsphere Q and S Resin specifications.

Property	UNOsphere Q	UNOsphere S
Type of ion exchanger	Strong anion	Strong cation
Functional group	$-N^{+}(CH_3)_3$	$-SO_3^{-}$
Median particle size	120 µm	80 µm
Total ionic capacity	120 µeq/ml Ni^{2+}	260 µeq/ml
Dynamic binding capacity* At 150 cm/hr At 600 cm/hr	 180 mg BSA/ml 125 mg BSA/ml	 60 mg IgG/ml 30 mg IgG/ml
Shipping counterion	Cl^{-}	Na^{+}
Recommended linear flow rate	50–1,200 cm/hr	50–1,200 cm/hr
Chemical compatibility 1.0 M NaOH (20°C) 1.0 M HCl (20°C)	 Up to 10,000 hr Up to 200 hr	 Up to 10,000 hr Up to 200 hr
pH stability	1–14	1–14
Antimicrobial agent	20% ethanol	20% ethanol
Regeneration	1–2 M NaCl	1–2 M NaCl
Storage conditions	20% ethanol or 0.1 M NaOH	20% ethanol or 0.1 M NaOH

* 10% breakthrough capacity determined with 2.0 mg/ml BSA (UNOsphere Q) 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 120 cm/hr, 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. For other regeneration methods, refer to section 6.

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 UNOsphere 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 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) for the 1 ml column or 6.0 ml/min (288 cm/hr) 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 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 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 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 Columns are available in 1 and 5 ml formats. UNOsphere Q and S 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

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, a result that should be expected due to the nature of the sample mixture. The following cleaning and regeneration procedure may be used. However, we recommend that you dispose of a column after several uses. To avoid cross-contamination, we recommend that each column be designated for use with a single protein. To maintain good flow properties, we recommend that the columns be cleaned between uses.

Acceptable CIP agents include 25% acetic acid, 8 M urea, 1% Triton X-100, 6.0 M potassium thiocyanate, 70% ethanol, 30% isopropyl alcohol, 1.0 M HCl, 1.0 M NaOH, and 6.0 M guanidine hydrochloride. Run the cleaning protocol at 2 ml/min for 1 ml columns. For 5 ml columns, run the cleaning protocol at 5 ml/min.

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 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 is in flowthrough	Optimize binding condition
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 UNOsphere Q and S Columns	
12009307	**EconoFit UNOsphere Q Column**, 1 x 1 ml column
12009301	**EconoFit UNOsphere Q Columns**, 5 x 1 ml columns
12009302	**EconoFit UNOsphere Q Column**, 1 x 5 ml column
12009303	**EconoFit UNOsphere Q Columns**, 5 x 5 ml columns
12009308	**EconoFit UNOsphere S Column**, 1 x 1 ml column
12009304	**EconoFit UNOsphere S Columns**, 5 x 1 ml columns
12009305	**EconoFit UNOsphere S Column**, 1 x 5 ml column
12009306	**EconoFit UNOsphere S Columns**, 5 x 5 ml columns
UNOsphere Q and S Resins	
1560101	**UNOsphere Q Resin**, 25 ml bottle
1560103	**UNOsphere Q Resin**, 100 ml bottle
156-0105	**UNOsphere Q Resin**, 500 ml bottle
156-0107	**UNOsphere Q Resin**, 10 L bottle
1560111	**UNOsphere S Resin**, 25 ml bottle
1560113	**UNOsphere S Resin**, 100 ml bottle
156-0115	**UNOsphere S Resin**, 500 ml bottle
156-0117	**UNOsphere S Resin**, 10 L bottle

Section 9
Bibliography

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