EconoFit Affi-Prep Protein A Chromatography Columns, 1 and 5 ml

Instruction Manual

Catalog #12009236
12009237

Please read the instructions in this manual prior to using EconoFit Affi-Prep Protein A Chromatography Columns, 1 and 5 ml.
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 Affi-Prep Protein A 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 Bio-Rad's Affi-Prep Protein A Resin. Affi-Prep Protein A Resin consists of highly purified Protein A covalently coupled to a unique macroporous polymer matrix. This support is intended for use in medium- to high-pressure chromatographic applications. All specifications have been developed using the Affi-Prep Protein A MAPS II Buffers (catalog #1536164).

The usefulness of Protein A purification for murine monoclonal antibodies has been limited because retention of most IgG_1 species represents a significant purification problem with published methods. The MAPS buffer system for Protein A affinity chromatography was developed to optimize the binding and recovery of many immunoglobulins, especially mouse monoclonal antibodies. The MAPS buffer system has been shown to increase Protein A's capacity for IgGs and IgMs from many different species. Approximately 50% of all IgMs will bind to Protein A when the MAPS buffer system is used. This buffer system is recommended for use with all Protein A affinity supports.

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), ion exchange, affinity, mixed-mode, and hydrophobic interaction chromatography. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of products in the EconoFit Column portfolio.

See Table 1 for the EconoFit Affi-Prep Protein A Column information and technical specifications.

Table 1. EconoFit Affi-Prep Protein A Column information and technical 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)
Fittings	10-32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Shipping solution	0.05 M sodium phosphate, pH 7.5 + 0.05% sodium azide
Storage conditions	As above
Autoclavability	Not autoclavable
Shelf life	1 year at 4°C
Monoclonal antibody loading capacity	Mouse IgG_1 = 8–10 mg/ml IgG_2a = 13–15 mg/ml IgG_2b = 13–15 mg/ml IgG_3 = 8–10 mg/ml
Polyclonal antibody loading capacity	Rat, sheep, bovine, equine, goat, rabbit, canine, and porcine IgG = 9–16 mg/ml; IgM = 5–7 mg/ml
Chemical stability	pH 2–14 1 N NaOH 8 M urea 50% methanol 6 M guanidine-HCl

Affi-Prep Protein A Resin is also available in bottles. Refer to ordering information in Section 8 of this manual. Go to **bio-rad.com/ResinsandColumns** for more information.

Section 3
Sample Preparation

For best results, and increased column life, samples and buffers should be degassed and filtered through a 0.45 µm filter.

1. Proper adjustment of the pH and ionic strength of the sample is critical for optimal binding. For best results, the sample pH should be adjusted to 9.0, and the ionic strength of the sample should approach that of the MAPS binding buffer. This can be achieved by sample dilution, dialysis, or buffer exchange using the Econo-Pac 10DG Desalting Columns or Bio-Gel P-6DG Gel Filtration Gel.
2. Ascites fluid should be diluted 1:2 with binding buffer. Higher concentrations of binding buffer can enhance the binding of low-affinity antibodies.
3. Tissue culture supernatant should be concentrated to approximately 5 mg immunoglobulin per ml, and then diluted 1:2 with binding buffer. For large volume samples where further dilution is not desired, we recommend adding the dry binding buffer salts directly to the sample instead of diluting the sample with prepared buffer.

Section 4
Preparing a Column and Subsequent Purification

1. Equilibrate the column with 5–10 bed volumes of Affi-Prep MAPS II Binding Buffer. After equilibration, the pH of the column effluent should be equal to the pH of the binding buffer (pH 9.0).
2. Apply the prepared sample to the column.
3. Wash the column with 10–15 bed volumes of binding buffer to remove all of the unbound contaminating components.
4. Elute the immunoglobulin with 5 bed volumes of Affi-Prep MAPS II Elution Buffer. Elute with an additional 10 bed volumes of elution buffer to ensure removal of all immunoglobulin.
5. Neutralize the eluted sample immediately after elution with 1 M Tris-HCl. (Avoid prolonged exposure of the purified immunoglobulin fraction to acidic pH.)
6. Regenerate the Affi-Prep Protein A Column with 50% methanol after every use. The column can be washed with 0.1 N NaOH every five to ten runs for a more stringent column wash. This NaOH wash should be used only after the regular methanol regeneration step. For complete sanitation (that is, removal of endotoxins and DNA), the support can be washed with 1.0 N NaOH. This is an acceptable method of sanitization for FDA purposes.
7. If the column will be reused right away, re-equilibrate it with at least 5 column volumes of MAPS binding buffer. If the column is to be stored, equilibrate it with a mild neutral buffer such as 0.05 M sodium phosphate, pH 7.5, containing 0.02–0.05% sodium azide.

Section 5
Scaling Up

EconoFit Affi-Prep Protein A Columns are available prepacked in 1 and 5 ml formats. The resin is also available in 5 and 25 ml bottles 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. 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 columns between uses.

Affi-Prep Protein A Columns should be regenerated with 50% methanol after every use. The column can be washed with 0.1 N NaOH every 5–10 runs for a more stringent wash.

The column can be sanitized in up to 1 N NaOH. Owing to the higher viscosity of concentrated NaOH solutions, the flow rate may need to be lowered to avoid overpressure issues.

After washing them with deionized water, EconoFit Columns should be purged and stored at room temperature in 0.05 M sodium phosphate, pH 7.5, containing 0.02–0.05% sodium azide.

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
	Target is 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 Affi-Prep Protein A Columns	
12009236	**EconoFit Affi-Prep Protein A Columns**, 5 x 1 ml prepacked affinity columns, 7 x 25 mm, max pressure 72 psi
12009237	**EconoFit Affi-Prep Protein A Column**, 1 x 5 ml prepacked affinity column, 16 x 25 mm, max pressure 72 psi
Affi-Prep Protein A Resin Bottles and Buffers	
1560006	**Affi-Prep Protein A Resin**, 5 ml
1560005	**Affi-Prep Protein A Resin**, 25 ml
1536164	**Affi-Prep Protein A MAPS Buffers**, 1.5 L binding and 1.1 L elution buffers
1536161	**Affi-Prep Protein A MAPS II Binding Buffer**, 5 ml

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