Econo-Pac®
Protein A Cartridge

Instruction Manual

Catalog Number
732-0093

BIO-RAD

Table of Contents

Section 1
Introduction

The Econo-Pac cartridges are a series of patented*, easy-to-use, prepacked chromatographic cartridges for fast, reproducible chromatographic separations. Cartridges are available for a variety of chromatographic techniques including gel filtration, ion exchange, affinity, and hydrophobic interaction. See Ordering Information for a listing of the complete Econo-Pac cartridge product line.

The patented design of the Econo-Pac cartridges offers:

- Resilient frits which minimize expansion or contraction of the chromatographic bed during a gradient run.
- Tapered construction for optimal elution.
- Manifold distribution chambers for improved sample and buffer distribution over the cross-sectional area of the cartridge.
- Luer-lock fittings for snap-on connection to any chromatography system or directly to a syringe.

The Econo-Pac protein A cartridge is packed with the Affi-Prep® protein A affinity chromatography support. This support is based on a spherical, rigid polymer with a narrow particle size distribution, which allows excellent resolution, high capacity, and high flow rates. These affinity chromatography cartridges are used for small scale purification of monoclonal antibodies (up to 7 mg per run). Detailed product information is given in Table 1.

Table 1. Description of Econo-Pac Protein A Cartridge

Type	Affinity support
Functional group	Protein A
Bed volume	1 ml
Antibody binding capacity	~7 mg mouse monoclonal antibody (IgG_1) ~14 mg human IgG
Particle diameter (nominal)	50 µm
Pore size (nominal)	1,000 Å

Recommended flow rate	0.1-0.5 ml/min (refer to section 4.3)
Maximum flow rate	6 ml/min
Operating pH range	2-14
Average back pressure	21 psi at 6 ml/min (MAPS II binding buffer at 20 °C)
Maximum operating pressure**	3.45 bar (50 psi or 345 KPa) at 20 °C
Cartridge and frit construction	Polypropylene
Shipping conditions	Semi-dry
Recommended storage	50 mM phosphate (pH 7.0), with 0.05% NaN_3

Section 2
Connecting to Bio-Rad's Econo System

The Econo-Pac protein A cartridge is ideal for use with Bio-Rad's Econo System, a low pressure chromatography system. The cartridge can be conve-

niently connected directly to the system using the Luer-lock fittings on the cartridge.

1. Install 0.8 mm ID tubing in the Model EP-1 Econo Pump.
2. To maximize gradient accuracy and apply samples efficiently, install 0.8 mm ID tubing from the pump to the Model MV-6 Injector Valve. For large sample volumes, we recommend using the Model EV-1 Econo Buffer Selector.
3. Connect the inlet of the cartridge to the male Luer-lock fitting on the Model MV-6 valve. Older units of the Model MV-6 valve do not have a male Luer-lock fitting. In this case, use a male-to-male Luer fitting from the Model MV-6 valve to the cartridge. For optimum performance, a cartridge should be mounted vertically with the arrow on the cartridge pointing downward.
4. Connect the cartridge outlet to the Model EM-1 Econo UV Monitor optics module using a short length (approximately 10 cm) of 0.8 mm ID tubing, and female and male Luer fittings provided in the tubing kit supplied with the Econo System.

Section 3
Connecting to Other Liquid Chromatography Systems

The Econo-Pac cartridges can be connected to any liquid chromatography system, provided that the maximum pressure limit (3.45 bar, 50 psi, or 345 KPa) of the cartridges is not exceeded. It is recommended that the system pressure limit be set according to the cartridge pressure limit. Pressures in excess of 3.4 bar are usually caused by restrictions in tubing or detector cells downstream from the cartridge. Bio-Rad offers two fittings kits for easy connection of an Econo-Pac cartridge to HPLC or FPLC -type systems.

3.1 HPLC Systems

The Econo-Pac cartridge HPLC adaptor fittings kit, catalog number 732-0112, provides fittings necessary to connect the cartridge to nut and ferrule type fittings found on most HPLC systems.

Alternatively, the cartridge can be connected to HPLC systems via a low dead volume ⅛ inch union with a new piece of stainless steel tubing attached to

the union. Simply slip a short length of the 0.8 mm ID tubing over 1/16 inch OD stainless steel tubing to a distance of 1 cm.

3.2 FPLC Systems

The Econo-Pac cartridge FPLC adaptor fittings kit, catalog number 732-0111, provides fittings necessary to connect the cartridge to the Omni-style fittings found on FPLC or related systems.

Alternatively, connection can be made by using two Upchurch P-621, ¼-28 to metric adaptors, one Upchurch P-619, ¼-28 to male Luer and one Upchurch P-628, ¼-28 to female Luer. Assemble the Luers to the ¼-28 metric adaptors. Attach the adaptor with the male Luer to the column inlet line of the FPLC system and the one with the female Luer to the FPLC column out line.

To prevent tubing or cartridge failure, the flow rate of HPLC or FPLC systems must not exceed maximum recommended flow rate for the cartridge.

Section 4
Preparing a Cartridge for Use

The Econo-Pac protein A cartridge is packed using 0.05 M sodium phosphate (pH 7.0) containing 0.05% sodium azide and shipped in a semi-dry condition to maximize shelf life. The air present in the cartridge is easily removed when preparing the cartridge for use. After connecting the cartridge to a liquid chromatography system, condition it as instructed below:

1. Set pump flow rate to 2.0 ml/min.†
2. Wash the cartridge with a degassed low salt buffer (such as 0.5 M sodium phosphate or MAPS II elution buffer) for 3 min.
3. Wash the cartridge with a degassed high salt buffer (such as 1.5 M sodium phosphate or MAPS II binding buffer) for 10 min. A small amount of air may remain just above the upper frit and in the inlet nozzle of the cartridge. Invert the cartridge so that the arrow points upward, allowing air to be

expelled into the cartridge and out through the outlet nozzle.

4. Equilibrate the cartridge for 10 min at 2 ml/min.
5. Invert the cartridge so that the cartridge arrow points downward.
6. Reduce the flow rate to 0.5 ml/min.

† When using a cartridge on HPLC, FPLC, or other high pressure systems, consider the maximum pressure rating for the cartridge when adjusting the flow rate.

4.1 Sample Preparation

Proper adjustment of the pH and ionic strength of the sample is critical for optimal binding. For best results, both the sample pH and ionic strength should be high. This is best accomplished with the MAPS II binding buffer, although other buffer systems may be used. (We will refer to the MAPS II buffers throughout the manual. Protocols for other buffer systems must be determined empirically.) Adjustment of the pH and ionic strength of the sample 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 Econo-Pac P6 cartridge, Bio-Spin® 6 or Bio-Spin 30 columns, Econo-Pac 10DG desalting columns, or Bio-Gel® P-6DG gel filtration gel. The choice of product will depend on the sample volume.

Table 2. Products for Buffer Exchange

Sample Volume	Recommended Product	Use	Catalog No.
50-100 µl	Bio-Spin 6 column	Desalting proteins ≥ 6 kD	732-6000
50-100 µl	Bio-Spin 30 column	Desalting proteins ≥ 30 kD	732-6004
100 µl-3 ml	Econo-Pac P6 cartridge	Desalting proteins ≥ 6 kD	732-0011
Up to 3 ml	Econo-Pac 10DG desalting columns	Desalting proteins ≥ 6 kD	732-2010
Unlimited	Bio-Gel P-6DG gel	Desalting proteins ≥ 6 kD	150-0738

Ascites fluid should be diluted 1:2 with MAPS II binding buffer. Higher concentrations of binding buffer can enhance the binding of low affinity antibodies.

Tissue culture supernatants may be concentrated to approximately 5 mg of immunoglobulin per ml, and then diluted 1:2 with MAPS II binding buffer. For large volume samples where further dilution is not desired, we recommend adding the dry MAPS II binding buffer salts directly to the sample (31.4 g buffer salts/100 ml sample) instead of diluting the sample with prepared buffer.

All samples should be filtered through a 0.45 µm filter.

4.2 MAPS II Buffers

The MAPS II buffers provide a dramatic improvement in protein A affinity methods for the purification of mouse IgG_1 antibodies from ascites fluid. Capacity is significantly increased to 8-10 mg IgG_1 per ml of support, which is 8-10 times higher than that obtained with published methods. [Ey, P. L., Prowse, S. J., and Jenkins, C. R., *Immunochemistry*, **15**, 429 (1978). Bigbee, W. L., Vanderlann, M., Fong, S. S. N., and Jensen, R. H., *Mol. Immunol.*, **20**, 1353 (1983).]

All subclasses of mouse IgG in addition to other species of IgG (human, rabbit, bovine, goat) can be purified with the MAPS buffers.

4.3 General Purification Protocol

Equilibrate the Econo-Pac protein A cartridge with 7 to 13 ml of MAPS II binding buffer and adjust the flow rate to a flow rate suitable for antibody binding (0.1 to 0.5 ml/min recommended). Binding efficiency of immunoglobulin to the 1 ml Econo-Pac protein A cartridge may increase with a slower flow rate, as demonstrated in Figure 1.

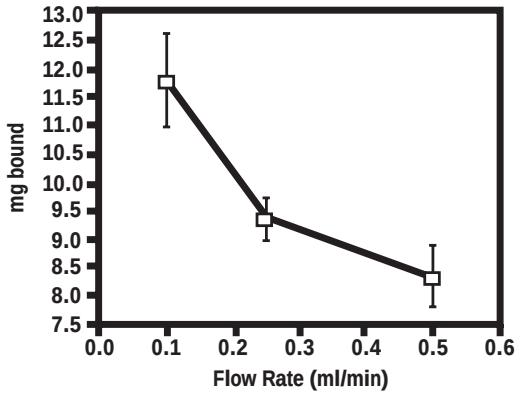

Fig. 1. Effect of flow rate on binding of bovine gamma globulin to 1 ml Econo-Pac protein A cartridges.

After adjusting the flow rate, apply the prepared sample to the cartridge. Wash the cartridge with 10-20 ml of binding buffer to remove all of the unbound contaminating components.

Elute the immunoglobulin with 5-10 ml of MAPS II elution buffer. Elute with an additional 10 ml of elution buffer to insure total removal of immunoglobulin.

Neutralize the eluted sample immediately after elution with 1 M Tris-HCl, pH 8.8, or 1 N NaOH. Prolonged exposure of the purified immunoglobulin to acid pH should be avoided.

Regenerate the Econo-Pac protein A cartridge with 7 ml of 50% methanol after each use and equilibrate with 7 to 13 ml of binding buffer if the cartridge is to be used. The pH of the cartridge effluent should be 9.0 when equilibrated.

4.4 Scaling Up the Separation

For quick scale up, two or three cartridges can be connected in series. Econo-Pac cartridges are also available in 5 ml format.

The Affi-Prep protein A support is available in larger amounts, from 25 ml to bulk quantities, for scaling up methods developed using the cartridges. In

addition, Bio-Rad carries an extensive line of empty chromatography columns.

Section 5
Care of the Cartridge

5.1 Cleaning

After 5-10 uses, an Econo-Pac protein A cartridge may require thorough cleaning and regeneration to remove bound contaminants. Most bound contaminants may be removed by following the procedure below:

1. Wash the cartridge with 7 ml of 50% methanol at 0.5-1.0 ml/min.†
2. Wash the cartridge with 5-8 ml of 0.1 N NaOH.
3. Equilibrate the cartridge with at least 7 ml of binding buffer.

The 0.1 N NaOH wash will remove any bound phenol red from the cartridge. For complete sanitation (i.e. removal of endotoxins and DNA) the cartridge can be washed with 1.0 N NaOH. This is an acceptable method of sanitation for FDA purposes. Follow

the procedure for Cleaning the Cartridge but substitute 1.0 N NaOH in step 2.

† When using a cartridge on HPLC, FPLC, or other high pressure systems, please consider the maximum pressure rating for the cartridge when adjusting the flow rate.

5.2 Storage

The Econo-Pac protein A cartridges should be stored in 0.05 M sodium phosphate buffer (pH 7.0) containing 0.05% NaN_3. Wash the cartridge with deionized water, then purge it with storage buffer.

Section 6
Technical Assistance

For additional information and technical assistance, contact your local Bio-Rad representative as listed on the back cover of our catalog, or, in the U.S.A., call Technical Service at 1-800-4BIORAD.

Section 7
Ordering Information

Catalog Number	Product Description	Type
732-0093	**Econo-Pac Protein A Cartridge, 5 x 1 ml**	Affinity
732-0091	**Econo-Pac Protein A Cartridge, 1 x 5 ml**	Affinity
153-6164	**Affi-Prep Protein A MAPS II Buffers**	Makes 1.5 L binding buffer and 1.5 L elution buffer
153-6161	**Affi-Prep Protein A MAPS II Binding Buffer**	Makes 5 L
153-6162	**Affi-Prep Protein A MAPS II Elution Buffer**	Makes 5 L
Other Econo-Pac Cartridges***		
732-0023	**Econo-Pac Q Cartridge, 5 x 1 ml**	Strongly basic anion exchanger
732-0003	**Econo-Pac CM Cartridge, 5 x 1 ml**	Weakly acidic cation exchanger
732-0063	**Econo-Pac S Cartridge, 5 x 1 ml**	Strongly acidic cation exchanger

Catalog Number	Product Description	Type
732-0053	**Econo-Pac Methyl HIC Cartridge, 5 x 1 ml**	Hydrophobic interaction
732-0083	**Econo-Pac HTP Cartridge, 5 x 1 ml**	Spherical hydroxylapatite
Bulk Quantities of Protein A Affinity Support		
156-0005	**Affi-Prep Protein A Support, 25 ml**	

Larger package sizes are available.

Fittings Kits

732-0111	**Econo-Pac Cartridge - FPLC Adaptor Fittings Kit**	
732-0112	**Econo-Pac Cartridge - HPLC Adaptor Fittings Kit**	

* US Patent 4,871,463

** Pressure limitation is for the cartridge. The Affi-Prep Protein A support stable to pressures up to 68 bar (1,000 psi or 6,800 KPa).

*** All Econo-Pac cartridges are also available in a 5 ml cartridge format. Larger package sizes for media are available for process scale chromatography. Contact your local Bio-Rad representative.

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