Econo-Pac®
Heparin Cartridge

Instruction Manual

Catalog Number
732-0071
732-0075

BIO-RAD

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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, hydroxyapatite, 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:

- 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.
- Resilient frits which minimize expansion or contraction of the chromatographic bed during a gradient run.
- Tapered construction for optimal elution.

The Econo-Pac heparin cartridge is packed with the Affi-Prep® heparin affinity chromatography support. This support is based on a spherical, rigid polymer with a narrow particle size distribution, which allows excellent resolution and high flow rates. This affinity chromatography cartridge is used for the small scale purification of growth factors, proteases, DNA-binding proteins, lipoproteins, endonucleases, and other biomolecules.

Table 1. Description of Econo-Pac Heparin Cartridge

Type	Affinity
Functional group	Covalently bound heparin
Bed volume	5 ml
Heparin content	Approximately 5 mg
Heparin activity	>50 units/ml based on antithrombin III assay
Particle diameter (nominal)	50 µm
Pore size (nominal)	1,000 Å
Recommended flow rate	1-3 ml/min
Maximum flow rate	6 ml/min
Operating pH range	2-12
Average back pressure	140 mbar (2 psi) at 6 ml/min (water at 20 °C)
Maximum operating pressure**	3.4 bar (50 psi or 0.36 mPa)

Cartridge and frit construction	Polypropylene
Shipping conditions	Semi-dry
Recommended storage	20 mM sodium phosphate buffer, pH 8.0, with 0.01% NaN3 at 4 °C.

Section 2
Connecting to Bio-Rad's Econo System

The Econo-Pac heparin cartridge is ideal for use with Bio-Rad's Econo System, a low pressure chromatography system. It can be conveniently connected directly to the system using the Luer-lock fittings in the cartridge.

1. Install 1.6 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.
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 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 (3.4 bar, 50 psi, or 0.36 mPa) of the cartridges is not exceeded. It is recommended 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 1/16 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, 1/4"-28 to metric adaptors, one Upchurch P-619, 1/4"-28 to male Luer and one Upchurch P-628, 1/4"-28 to female Luer. Assemble the Luers to the 1/4"-28 to 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 outlet 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 heparin cartridge is packed in buffer containing 0.01% sodium azide and is 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 degassed low salt buffer for 2 minutes at 2 ml/min.
3. Wash the cartridge with degassed high salt buffer for 10 minutes at 6 ml/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. Wash the cartridge with low salt buffer for 10 minutes at 6 ml/min.†
5. Invert the cartridge so that the cartridge points downward.
6. Reduce the flow rate to 2.0 ml/min.†

4.1 Sample Preparation

No sample preparation is required.

4.2 General Purification Protocol

Heparin affinity chromatography is usually performed using increasing salt gradients or pH gradients to elute the sample components. For best results, and increased cartridge life, buffers should be degassed and filtered through a 0.45 µm filter. Common buffers for heparin affinity chromatography are phosphate buffered saline and 2.0 M NaCl, 100 mM sodium phosphate, pH 6.8.

An appropriate starting point for separation of many samples is a linear gradient from 0 to 2.0 M NaCl over 20 minutes at a flow rate of 2.0 ml per minute. The sep-

aration can then be optimized by changing the flow rate and gradient profile.

At the end of each run, the cartridge can be regenerated at 6 ml/min† with 10-20 ml of buffer containing 2.0 M NaCl. Follow this with 20 ml of starting buffer. Return to the desired flow rate and proceed with the next separation.

4.3 Scaling Up the Separation

For quick scale up, two or three cartridges can be connected in series. For scaling up methods developed using the cartridges, inquire about the availability of larger quantities of Affi-Prep heparin affinity chromatography support, and Bio-Rad's line of empty chromatography columns.

Section 5
Care of the Cartridge

5.1 Cleaning

After repeated use, a heparin affinity chromatography cartridge may require thorough cleaning and regeneration

to remove bound contaminants. Bound contaminants may be removed by following the procedure below:

1. Wash the cartridge with 50 ml of deionized water (6 ml/min).†
2. Wash the cartridge with 20-30 ml of 100 mM NaOH (1 ml/min).
3. Wash with 50 ml of deionized water (6 ml/min).†
4. Wash with 50 ml high salt buffer (6 ml/min).†
5. Equilibrate with 25 ml low salt buffer (6 ml/min).†
6. Reduce the flow to 2 ml/min.

5.2 Sanitizing

The Econo-Pac heparin cartridge can be sanitized with 100 mM NaOH. After sanitizing, sterility may be maintained by using sterile buffers. A sanitized cartridge should be prepared as described in the Preparing a Cartridge For Use section of this manual.

5.3 Storage

The Econo-Pac heparin cartridge should be stored at 4 °C in low ionic strength buffer containing 0.01%

Catalog Number	Product Description	Type
732-0011	**Econo-Pac P6 Cartridge,** 1	Size exclusion,
732-0015	**Econo-Pac P6 Cartridge,** 5	desalting
732-0081	**Econo-Pac HTP Cartridge,** 1	Hydroxylapatite
732-0085	**Econo-Pac HTP Cartridge,** 5	
732-0091	**Econo-Pac Protein A Cartridge,** 1	Affinity

Fittings Kits

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

Many Econo-Pac Cartridges are available in both 5 ml and 1 ml sizes. Inquire about bulk quantities of Affi-Prep heparin support.

* US Patent 4,871,463

** Pressure limitation is for the cartridge. The Affi-Prep supports are stable to pressures up to 68 bar (1,000 psi).

† If the cartridge is being used on a system other than the Econo System or other low pressure workstation, consider maximum pressure ratings for the cartridge when adjusting the flow rate.

FPLC is a registered trademark of Pharmacia.

NaN_3. Wash the cartridge with deionized water, then purge it with the 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.

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
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