Econo-Pac®
Methyl and t-Butyl HIC Cartridges, 5 ml

Instruction Manual

Catalog Numbers
732-0051, 732-0055
732-0056, 732-0057

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, hydrophobic interaction, and hydroxylapatite chromatography.

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 methyl HIC cartridge is packed with Macro-Prep® methyl HIC hydrophobic interaction chromatography support. The Econo-Pac t-Butyl HIC cartridge is packed with Macro-Prep t-Butyl HIC support. These supports are based on a spherical, rigid polymer with a narrow particle size distribution, which provides excellent resolution and high flow rates. The hydrophobic interaction chromatography cartridge is used for the small scale purification of biomolecules. Detailed product technical data and chromatographic characteristics are given in Table 1.

Table 1. Description of the Econo-Pac HIC Cartridges

	Econo-Pac Methyl HIC cartridge	Econo-Pac t-Butyl HIC cartridge
Type	Hydrophobic interaction	Hydrophobic interaction
Functional group	$-OCH_3$	$-\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{C}}}}-CH_3$
Bed volume	5 ml	5 ml
Protein binding capacity	≥ 110 mg human serum albumin dissolved in 2.4 M ammonium sulfate,100 mM sodium phosphate, pH 6.8	≥ 65 mg human serum albumin, identical conditions

	Econo-Pac Methyl HIC cartridge	Econo-Pac t-Butyl HIC cartridge
Particle diameter (nominal)	50 µm	50 µm
Pore size (nominal)	1,000 Å	1,000 Å
Recommended flow rate	0.5-3.0 ml/min	0.5-3.0 ml/min
Maximum flow rate	6 ml/min	6 ml/min
Operating pH range	2-12	2-12
Maximum operating pressure**	3.4 bar (50 psi) at 20 °C.	
Cartridge and frit construction	Polypropylene	Polypropylene
Shipping conditions	Semi-dry	Semi-dry
Recommended storage	50 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 0.05% NaN_3	

Section 2
Connecting to Bio-Rad's Econo Systems

Econo-Pac HIC cartridges are ideal for use with Bio-Rad's Econo System, a low pressure chromatography system. The cartridge can be conveniently connected directly to the system using the Luer-lock fittings on 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 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 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 metric adaptors. Attach the adaptor with the male

Luer to the column inlet line of the FPLC system and the one with the female 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

Econo-Pac Methyl and t-Butyl HIC cartridges are packed using a buffer consisting of 50mM Tris-HCl, pH 8.0, 0.1 M NaCl, and 0.05% NaN_3, and are shipped in a semi-dry condition to maximize shelf life. Any air present in the cartridge is easily removed when preparing the cartridge for use. After connecting the cartridge to a liquid chromatography system, prepare 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. Equilibrate the cartridge with high salt buffer for 2 minutes at 6 ml/min.
6. Invert the cartridge so that the cartridge points downward.
7. Reduce the flow rate to that being used for the separation.

4.1 Sample Preparation

Proper adjustment of the sample pH and ionic strength is critical for consistent and reproducible results in all chromatographic techniques. For best results with hydrophobic interaction chromatography, the sample should be adjusted to the concentration of the starting buffer by dissolving salt in the sample, by mixing the sample with a concentrated solution of the desired salt, or by dialyzing the sample against the starting buffer.

4.2 General Purification Protocol

Hydrophobic interaction chromatography is usually performed using decreasing salt gradients to elute the sample components. For best results, and increased cartridge life, both samples and buffers should be degassed and filtered through a 0.45 µm filter. Common buffers for hydrophobic interaction chromatography are 2.4 M ammonium sulfate in 100 mM sodium phosphate, pH 6.8, and 100 mM sodium phosphate, pH 6.8.

An appropriate starting point for separation of many samples is a linear gradient from 2.4 M to 0 M ammonium sulfate over 30 minutes at a flow rate of 2.0 ml per minute. The separation can then be optimized by changing the flow rate and gradient profile.

At the end of each run, the cartridge can be regenerated at higher flow rates for the next run by rinsing with 15 ml of 100 mM sodium phosphate buffer and equilibrating with 15 ml of the 2.4 M ammonium sulfate solution. 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 of the same type can be connected in series. For scaling up methods developed using the cartridges, inquire about the availability of larger quantities of Macro-Prep HIC hydrophobic interaction chromatography supports, and Bio-Rad's line of empty chromatography columns.

Section 5
Care of the Cartridge

5.1 Cleaning the Cartridge

After repeated use, a hydrophobic interaction 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 (6ml/min).
2. Wash the cartridge with 20-30 ml of the 100 mM NaOH (1 ml/min).

3. Wash with 50 ml of deionized water (6 ml/min).
4. Wash with 50 ml of 20% glycerol (2 ml/min).
5. Wash with 50 ml of deionized water (6 ml/min).
6. Wash with 50 ml low salt buffer (6 ml/min).
7. Equilibrate with 25 ml high salt buffer (6 ml/min).
8. Reduce the flow to 2 ml/min.

If bound contaminants, such as dyes or detergents, persist after following the procedure above, use the alternative procedure below:

5.2 Wash Alternative

Perform steps 1-6 above. Wash with 50 ml of 50% propanol/water solution. Then continue with steps 5-8 above.

5.3 Autoclaving

The Econo-Pac HIC cartridges can be autoclaved at 121 °C, 2 bar, for 30 minutes. Loosen the end caps before autoclaving. After autoclaving, sterility may be maintained by using sterile buffers. An autoclaved cartridge should be prepared as described in the Preparing a Cartridge For Use section of this manual.

5.4 Storage

The Econo-Pac HIC cartridges should be stored in low ionic strength buffer containing 0.05% NaN_3 or in 20% v/v ethanol solution. Wash the cartridge with deionized water, then purge it with one of these solutions.

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
Ordering Information		
732-0051	**Econo-Pac Methyl HIC Cartridge,** 1 x 5 ml	Hydrophobic interaction
732-0055	**Econo-Pac Methyl HIC Cartridge,** 5 x 5 ml	Hydrophobic interaction
732-0056	**Econo-Pac t-Butyl HIC Cartridge,** 1 x 5 ml	Hydrophobic interaction
732-0057	**Econo-Pac t-Butyl HIC Cartridge,** 5 x 5 ml	Hydrophobic interaction
Other Econo-Pac Cartridges		
732-0026	**Econo-Pac High Q Cartridge,** 1 x 5 ml	Strongly basic anion exchanger
732-0027	**Econo-Pac High Q Cartridge,** 5 x 5 ml	Strongly basic anion exchanger
732-0028	**Econo-Pac High Q Cartridge,** 5 x 1 ml	Strongly basic anion exchanger
732-0066	**Econo-Pac High S Cartridge,** 1 x 5 ml	Strongly acidic cation exchanger

Catalog Number	Product Description	Type
732-0067	**Econo-Pac High S Cartridge,** 5 x 5 ml	Strongly acidic cation exchanger
732-0068	**Econo-Pac High S Cartridge,** 5 x 1 ml	Strongly acidic cation exchanger
732-0001	**Econo-Pac CM Cartridge,** 1 x 5 ml	Weakly acidic cation exchanger
732-0005	**Econo-Pac CM Cartridge,** 5 x 5 ml	Weakly acidic cation exchanger
732-0003	**Econo-Pac CM Cartridge,** 5 x 1 ml	Weakly acidic cation exchanger
732-0031	**Econo-Pac DEAE Blue Cartridge,** 1 x 5 ml	Dye affinity, weakly basic anion exchanger
732-0035	**Econo-Pac DEAE Blue Cartridge,** 5 x 5 ml	Dye affinity, weakly basic anion exchanger
732-0053	**Econo-Pac Methyl HIC Cartridge,** 5 x 1 ml	Hydrophobic interaction
732-0058	**Econo-Pac t-Butyl HIC Cartridge,** 5 x 1 ml	Hydrophobic interaction
732-0071	**Econo-Pac Heparin Cartridge,** 1 x 5 ml	Affinity
732-0075	**Econo-Pac Heparin Cartridge,** 5 x 5 ml	Affinity

Catalog Number	Product Description	Type
732-0081	**Econo-Pac HTP Cartridge,** 1 x 5 ml	Hydroxyapatite
732-0085	**Econo-Pac HTP Cartridge,** 5 x 5 ml	Hydroxyapatite
732-0083	**Econo-Pac HTP Cartridge,** 5 x 1 ml	Hydroxyapatite
732-0091	**Econo-Pac Protein A Cartridge,** 1 x 5 ml	Affinity
732-0093	**Econo-Pac Protein A Cartridge,** 5 x 1 ml	Affinity
732-0101	**Econo-Pac Blue Cartridge,** 1 x 5 ml	Affinity
732-0105	**Econo-Pac Blue Cartridge,** 5 x 5 ml	Affinity
732-0011	**Econo-Pac P6 Cartridge,** 1 x 5 ml	Desalting
732-0015	**Econo-Pac P6 Cartridge,** 5 x 5 ml	Desalting

Fittings Kits

732-0111 **Econo-Pac Cartridge-FPLC adaptor fittings kit**

732-0112 **Econo-Pac Cartridge-HPLC adaptor fittings kit**

- Larger package sizes of media are available for process scale chromatography. Inquire with your local Bio-Rad representative.

* US Patent 4,871,463

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

FPLC is a registered trademark of Pharmacia.

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