Econo-Pac® P6 Cartridge

Instruction Manual

Catalog Numbers
732-0081
732-0085

BIO-RAD

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LIT273 Rev B

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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, ion exchange, affinity, and hydrophobic interaction. See Product Information for a listing of the complete Econo-Pac cartridge product line.

The patented design of the Econo-Pac cartridges offers:

- 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.
- Manifold distribution chambers for improved sample and buffer distribution over the cross sectional area of the cartridge.

The Econo-Pac P6 cartridge can be used for rapid desalting and buffer exchange of simple and complex mixtures of macromolecules. The Econo-Pac P6 cartridge is packed with Bio-Gel P-6 gel, a neutral, hydrophilic support with an exclusion limit of 6,000 MW. The support consists of spherical, crosslinked polyacrylamide beads exhibiting a narrow particle size distribution, which allow excellent resolution and high flow rates. Detailed product technical data and chromatographic characteristics are given in Table 1.

Table 1. Description of Econo-Pac P6 Cartridge

Type	Size exclusion
Bed volume	5 ml
Void volume	1.8 ml
Sample capacity	100 µl – 3.0 ml
Particle diameter (nominal)	120 µm
Recommended flow rate	0.5 – 1.5 ml/min
Maximum flow rate	4.5 ml/min
Operating pH range	3 – 7.5
Average back pressure	280 mbar (4 psi) at 4.5 ml/min (1.0 M NaCl at 20 °C)
Maximum operating pressure**	680 mbar (10 psi) at 20 °C
Cartridge and frit construction	Polypropylene
Shipping conditions	Fully hydrated in 20 mM sodium phosphate buffer, pH 6, containing 0.02% NaN_3
Recommended storage	20 mM sodium phosphate buffer, pH 5.5 – 7.0, containing 0.02% NaN_3

Section 2
Connecting to Bio-Rad's Econo System

The Econo-Pac P6 cartridge is ideal for use with Bio-Rad's BioLogic Systems. It 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 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 the female and male Luer fittings provided in the tubing kit supplies with the Econo System.

Caution: Do not run the purge mode with the Econo-Pac P6 cartridge. When 1.6 mm ID tubing is installed in the Model EP-1 Pump, it delivers 6.4 ml/min flow rate when in the purge mode. The maximum recommended flow rate for the Econo-Pac P6 cartridge is 4.5 ml/min.

Section 3
Connecting to Other Liquid Chromatography Systems

The Econo-Pac cartridges can be connected to any liquid chromatography system, provided that the maximum flow rate (4.5 ml/min) and maximum operating pressure limit (680 mbar or 10 psi) of the

cartridges are not exceeded. Higher pressures are usually caused by restrictions in tubing or detector cells downstream from the cartridge.

3.1 HPLC Systems

The Econo System tubing kit, catalog number 731-8244, provides the fittings necessary to connect the cartridge to an HPLC system. Cut two pieces of 0.8 mm ID tubing to a length of 2.5 cm. Attach a male Luer-with-Barb to one piece and a female Luer-with-Barb to the other. Attach the piece with the male Luer to the inlet of the cartridge. Slip a red lock-ring onto the tubing, being certain that the bevel of the lock-ring faces away from the cartridge. Slip the other end of the tubing onto a 1/16 inch OD stainless steel tubing connected to the HPLC system injector valve. push the red lock-ring snugly against the ferrule on the stainless steel tubing. Similarly, connect the outlet of the cartridge to the stainless steel tubing leading to the detector.

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

To connect a cartridge to an FPLC medium pressure chromatography system, use two Upchurch P-621, 1/4-28 to metric adaptors, one Upchurch P-619, 1/4-28 to male Luer, and one Upchurch P-618, 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.

The flow rate of HPLC or FPLC systems must not exceed maximum recommended flow rate for the cartridge, to prevent tubing or cartridge failure. The Upchurch Luer fittings do not have Luer-lock features, and thus do not tolerate pressures in excess of 1 bar (15 psi).

Section 4
Syringe and Luer-lock 3-way Stopcock

To use the Econo-Pac P6 cartridge with syringes and a Luer-lock 3-way stopcock for desalting samples or exchanging samples into new buffers, refer to Figure A and the instructions in the With a Syringe section.

Section 5
Preparing a Cartridge For Use

The Econo-Pac P6 cartridges are packed using sterile buffer containing sodium azide and are shipped in a fully hydrated state to maximize shelf life.

After connecting the cartridge to a liquid chromatography system or a syringe, prepare it as instructed below. If air is accidentally introduced into a cartridge, it can be easily removed following these same instructions.

1. Wash the cartridge with 20 ml of degassed water or buffer at a rate of 4.0 ml/min.
2. 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.
3. Wash the cartridge with an additional 30 ml of water or buffer at a rate of 4.0 ml/min.
4. Invert the cartridge so that the cartridge points downward; and, if using a liquid chromatography system, reduce the flow rate to 1.0 ml/min.

Section 6
Sample Preparation

If the sample contains insoluble material, filter it with a 0.45 µm filter before applying it to the cartridge.

Section 7
Desalting and Buffer Exchange

Samples can be desalted or the sample buffer exchanged by connecting the cartridge to a liquid chromatography system or to a Luer-lock 3-way stopcock connected to syringes. The void volume for the Econo-Pac P6 cartridge is 1.8 ml. The desalted or the sample in its new buffer is contained within the fraction between 1.5 ml and 4.5 ml with the highest concentration of sample in the 1.8 ml to 3.0 ml fraction.

7.1 With a Liquid Chromatography System

1. Set the flow rate to the desired flow (0.5 to 1.5 ml/min).
2. Wash the prepared cartridge with 5 ml of the degassed buffer.
3. Inject up to 3.0 ml of sample, discarding the first 1.5 ml of effluent.

4. Monitor the cartridge effluent with a UV monitor or a conductivity monitor.

7.2 With a Syringe

The flow rate during sample application and collection should be controlled to between 0.5 and 1.5 ml/min when using a syringe. The buffer and sample syringes should be loaded before attaching them to the Luer-lock 3-way stopcock.

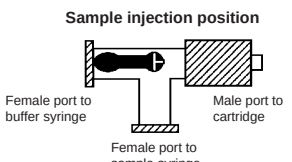

Fig. A. Luer-lock 3-way Stopcock.

Sample sizes between 100 µl and 1.5 µl

1. Connect a syringe containing the sample to the female port of a Luer-lock 3-way stopcock (catalog number 732-8103) as indicated in Figure A.
2. Connect a 20 ml syringe filled with degassed buffer to the female port as indicated in Figure A.
3. Clear air from the stopcock by first turning the valve to the sample injection position and pushing a small amount of sample into the stopcock. Then turn the valve to the buffer injection position and push enough buffer into the stopcock to clear remaining air.
4. Connect the prepared cartridge to the male port of the stopcock.
5. Turn the valve to sample injection position. Discard up to 1.5 ml of the effluent. If less than 1.5 ml of sample is injected, the remaining 1.5 ml of effluent to discarded will come from the next step.
6. Turn the valve to the buffer injection position. Discard the remaining effluent up to a total 1.5 ml which includes the volume of sample injected.

7. Collect the next 3.0 ml of effluent (the fraction between 1.5 ml and 4.5 ml). This fraction contains the desalted sample or the sample exchanged into the buffer.
8. Wash the cartridge with 15 ml of degassed buffer to prepare for the next sample application.

Samples sizes between 1.5 ml and 3.0 ml

1. Follow steps 1–4 above.
2. Turn the valve to sample injection position. Discard up to 1.5 ml of the effluent. Collect the remaining effluent.
3. Turn the valve to the buffer injection position and collect up to 3.0 ml of effluent, *i.e.* the fraction between 1.5 ml and 4.5 ml.
4. Wash the cartridge with 15 ml of degassed buffer to prepare for the next sample application.

Section 8
Autoclaving

The Econo-Pac P6 cartridge can be autoclaved at 121 °C, 2 bar, for 30 minutes in 0.05 M HEPES buffer, pH 6.2. Remove the inlet and outlet caps from the cartridge, then wash it with 25 ml of 0.05 M HEPES buffer, pH 6.2. Replace the caps, leaving them loose. Autoclave and cool. Tighten the caps.

Caution: Autoclaving Econo-Pac P6 cartridges or Bio-Gel P-6 gel in other buffers or in water may damage the support. To avoid damaging the support, use a buffer with a pH between 5 and 6.5, *e.g.* HEPES buffer at pH 6.2.

Section 9
Storage

The Econo-Pac P6 cartridge can be stored in the low ionic strength buffer containing 0.02% NaN_3 described in Table 1 or after autoclaving as described above. If storing the cartridge in a buffer containing azide, wash it with 25 ml of the buffer, attach the caps, and put it into storage.

Section 10
Scaling Up the Separation

For quick scale up, two to three cartridges can be connected in series. The Bio-Gel P-6 supports are also available in larger amounts, from 100 g to bulk quantities, for scaling up of methods developed using the cartridges. In addition, Bio-Rad carries an extensive line of empty chromatography columns.

Section 11
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 USA, call Technical Service at 1-800-4BIORAD.

Section 12
Product Information

Catalog Number	Product Description
	Econo-Pac size exclusion, desalting cartridges
732-0011	**Econo-Pac P6 Cartridge,** size exclusion, desalting, 1
732-0015	**Econo-Pac P6 Cartridge,** size exclusion, desalting, 5
	Other types of Econo-Pac cartridges
732-0051	**Econo-Pac HIC Cartridge,** hydrophobic interaction, 1
732-0055	**Econo-Pac HIC Cartridge,** hydrophobic interaction, 5
732-0071	**Econo-Pac Heparin Cartridge,** affinity, 1
732-0075	**Econo-Pac Heparin Cartridge,** affinity, 5
732-0031	**Econo-Pac DEAE Blue Cartridge,** dye affinity, weakly basic anion exchanger, 1
732-0035	**Econo-Pac DEAE Blue Cartridge,** dye affinity, weakly basic anion exchanger, 5
732-0101	**Econo-Pac Blue Cartridge,** dye affinity, 1
732-0105	**Econo-Pac Blue Cartridge,** dye affinity, 5

Catalog Number	Product Description
Bulk quantities of size exclusion, desalting supports	
150-0738	**Bio-Gel P-6DG gel,** 100 g
150-0739	**Bio-Gel P-6DG gel,** 1,000 g

* US Patent 4,871,463

** The maximum pressure limit for the cartridge is 3.4 bar (50 psi) at 20 °C.

FPLC is a registered trademark of Pharmacia.