



# **Econo-Pac<sup>®</sup> CHT-II Cartridge**

## **Instruction Manual**

**Catalog Number**  
**732-0083**

***BIO-RAD***

# Table of Contents

<b>Section 1</b>	<b>Introduction .....</b>	<b>1</b>
<b>Section 2</b>	<b>Connecting to Bio-Rad's Econo System .....</b>	<b>4</b>
<b>Section 3</b>	<b>Connecting to Other Liquid Chromatography Systems .....</b>	<b>5</b>
3.1	HPLC Systems .....	6
3.2	FPLC® Systems.....	6
<b>Section 4</b>	<b>Preparing a Cartridge For Use.....</b>	<b>7</b>
4.1	Sample Preparation .....	8
4.2	General Purification Protocol .....	9
4.3	Scaling Up the Separation.....	11
<b>Section 5</b>	<b>Care of the Cartridge .....</b>	<b>11</b>
5.1	Cartridge Regeneration .....	11
5.2	Storage .....	12
<b>Section 6</b>	<b>References.....</b>	<b>13</b>
<b>Section 7</b>	<b>Technical Assistance.....</b>	<b>14</b>
<b>Section 8</b>	<b>Ordering Information .....</b>	<b>15</b>

# 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, hydroxyapatite and hydrophobic interaction.

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 CHT-II cartridge is packed with a porous spherical hydroxyapatite support. This support is

composed of specially treated hydroxyapatite  $[\text{Ca}_5(\text{PO}_4)_3\text{OH}]_2$  with a narrow particle size distribution, which allows improved resolution, high capacity, and high flow rates. This hydroxyapatite chromatography cartridge is used for the purification of monoclonal antibodies,<sup>1-7</sup> proteins,<sup>8-9</sup> enzymes,<sup>10-12</sup> nucleic acids,<sup>13-18</sup> and viruses. For a discussion of the separation mechanism of hydroxyapatite, refer to the publication of M. Gorbunoff.<sup>19-21</sup> Detailed product information is given in Table 1.

**Table 1. Description of Econo-Pac CHT-II Cartridge**

<b>Type</b>	Inorganic support
<b>Functional group</b>	Hydroxyapatite, $[\text{Ca}_5(\text{PO}_4)_3\text{OH}]_2$
<b>Bed volume</b>	1 ml
<b>Protein capacity</b>	Bovine serum albumin, 3 mg [10 mM sodium phosphate (pH 6.8)] Lysozyme, 6 mg [10 mM sodium phosphate (pH 6.8)]
<b>DNA capacity</b>	Calf thymus, 160 $\mu\text{g}$ [10 mM sodium phosphate (pH 6.8)]

<b>Particle diameter (nominal)</b>	20 $\mu\text{m}$
<b>Pore size (nominal)</b>	800 $\text{\AA}$
<b>Recommended flow rate</b>	0.6-0.8 ml/min
<b>Maximum flow rate</b>	1.0 ml/min
<b>Operating pH range</b>	5.5-14
<b>Average back pressure</b>	18 psi at 0.6 ml/min (0.5 M sodium phosphate buffer, pH 6.8, at 20 °C). Typical range = 12-26 psi
<b>Maximum operating pressure</b>	3.45 bar (50 psi or 345 KPa) at 20 °C
<b>Cartridge and frit construction</b>	Polypropylene
<b>Autoclavability</b>	Cartridge is autoclavable in low salt buffer, pH 8, with end fittings loosened, for 30 minutes at 121 °C.
<b>Incompatibilities</b>	Ultra-pure water, EDTA (or other calcium chelators)
<b>Shipping conditions</b>	Moist

**Recommended storage**

100 mM phosphate (pH 7.0), with 0.02%  $\text{NaN}_3$  (see Section 5.2 for alternative storage conditions)

---

## Section 2 Connecting to Bio-Rad's Econo System

The Econo-Pac CHT-II cartridge is 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 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.
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<sup>®</sup>-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  $\frac{1}{16}$  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  $\frac{1}{16}$  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 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 CHT-II cartridge is packed using 0.5 M sodium phosphate (pH 6.8) containing 0.02% sodium azide and 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 0.8 ml/min.†
2. Wash the cartridge with degassed low salt (0.01 M phosphate) buffer for 3 minutes.

† 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.

3. Wash the cartridge with degassed high salt (0.5 M phosphate) buffer for 10 minutes. 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 15 minutes at 0.8 ml/min.
5. Invert the cartridge so that the cartridge arrow points downward.
6. Reduce the flow rate to 0.6-0.8 ml/min.

## 4.1 Sample Preparation

Proper adjustment of the pH and ionic strength of the sample is critical for optimal binding of proteins and nucleic acids. For best results, the sample pH should be adjusted to 7.2, and the ionic strength of the sample should approach that of the starting buffer. This 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 sample volume (see Table 2). All samples should be filtered through a 0.45 µm filter.

**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 P-6 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

## 4.2 General Purification Protocol

### Protein Separations

Equilibrate the Econo-Pac CHT-II cartridge with 5-10 ml of 10 mM sodium phosphate buffer (pH 7.2) at 0.8 ml/min. Reduce the flow rate to 0.6-0.8 ml/min and apply the prepared sample to the cartridge. Initiate the gradient. Sample components can be selectively eluted

from the cartridge by running a gradient from 10 mM to 400 mM sodium phosphate buffer, pH 6.8, over a period of 15-20 minutes. It is recommended that the final gradient conditions (high salt buffer) be held for another 5 minutes to completely elute bound material.

### **Nucleic Acid Separations**

At room temperature, equilibrate the Econo-Pac CHT-II cartridge with 5-10 ml of 10 mM sodium phosphate buffer (pH 7.2) at 0.8 ml/min. Reduce the flow rate to 0.6-0.8 ml/min and apply the prepared sample to the cartridge. Initiate the gradient. Sample components can be selectively eluted from the cartridge by changing both the phosphate concentration and the pH.

For both protein and nucleic acid procedures, it may be necessary to increase one or more of the following parameters to increase resolution of the two species: Temperature, detergent concentration, final phosphate concentration, duration of separation. Re-equilibrate the Econo-Pac CHT-II cartridge with 3 ml of starting buffer. To clean the cartridge, see Section 5.1.

## **4.3 Scaling Up the Separation**

For quick scale-up, two or three cartridges can be connected in series. Macro Prep Ceramic Hydroxyapatite type II is also available in bulk. In addition, Bio-Rad carries an extensive line of empty chromatography columns.

## **Section 5 Care of the Cartridge**

### **5.1 Cartridge Regeneration**

After 5 to 10 uses, an Econo-Pac CHT-II 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 5 ml of 0.1 M NaOH at 0.8 ml/min.<sup>†</sup>
2. Wash with 5 ml of elution buffer.

<sup>†</sup> 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.



3. Equilibrate the cartridge with at least 3 to 5 ml of starting buffer.

If the above method fails to regenerate the cartridge, 400 mM Na<sub>3</sub>PO<sub>4</sub> may help to remove contaminants, as it provides both higher ionic strength and high pH (about pH 12). The Econo-Pac CHT-II cartridge may also be cleaned with urea, guanidine, acetonitrile, and neutral detergents.

For complete sanitation (i.e. removal of endotoxins and DNA) the cartridge can be washed with 1.0 M NaOH. This is an acceptable method of sanitation for FDA purposes. Follow the procedure for Cartridge Regeneration, but substitute 1.0 M NaOH in step 2.

## 5.2 Storage

The Econo-Pac CHT-II cartridge may be stored in 0.1 M sodium phosphate buffer, pH 7.0, containing 0.02% NaN<sub>3</sub>. Alternatively, 5 mM phosphate buffer with 20-30% methanol or ethanol can be used. Either will help to eliminate microbial growth.

## Section 6 References

1. Stanker, L. D., Vanderlaan, M. and Juarez-Salinas, H., *J. Immunol. Methods*, **76**, 157 (1985).
2. Brooks, T. and Stevens, A., *American Laboratory*, **17**, 54, (1985).
3. Juarez-Salinas, H. et al., *Methods in Enzymology*, **131**, 615 (1986).
4. Juarez-Salinas, H., et al., Commercial Production of Monoclonal Antibodies, (Seaver, S., ed.) Marcel Dekker, New York.
5. Bukovsky, J. and Kennett, R., *Hybridoma*, Vol. 6, Number 2, Mary Ann Liebert, Inc. Publishers (1987).
6. Salinas, H. et al., *BioTechniques*, May-June (1984).
7. Smith, G., et al., *Anal. Biochem.*, **141**, 432 (1984).
8. Engel, W. D. S., Schagger, H. and Von Jagow, G., *Biochim. Biophys. Acta*, **592**, 211 (1980).
9. Köck, A. and Luger, T. A., *J. Chrom.*, **296**, 293 (1984).
10. Moseman McCoy, M. I., Lubben, T. H. and Gumpport, R. I., *Biochim. Biophys. Acta*, **562**, 149 (1979).
11. Tsang, A. S. and Coukell, M. B., *Eur. J. Biochem.*, **95**, 407 (1979).
12. Gunzburg, J., Part, D., Guiso, N. and Véron, M. *Biochem.*, **23**, 3805 (1984).
13. Kantler, P. M. and Swartz H. S., *Anal. Biochem.*, **97**, 77 (1979).
14. Beland, F. A. Dooley, K. L., and Casciano, D. A., *J. Chrom.*, **714**, 177 (1979).

15. Geck, P. and Nász, I., *Anal. Biochem.*, **135**, 264 (1983).
16. Genthner, F., Hook, L. and Strohl, W., *Applied and Environmental Microbiology*, **1007**, October 1985.
17. Vincent III, W. and Goldstein, E., *Anal. Biochem.*, **110**, 123 (1981).
18. Johnson, T. and Ilan, J., *Anal. Biochem.*, **132**, 20 (1983).
19. Gorbunoff, M. J., *Anal. Biochem.*, **136**, 425 (1984).
20. Gorbunoff, M. J., *Anal. Biochem.*, **136**, 433 (1984).
21. Gorbunoff, M. J., and Timasheff, S. N., *Anal. Biochem.*, **136**, 440 (1984).

## Section 7 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 8 Ordering Information

<b>Catalog Number</b>	<b>Product Description</b>	<b>Type</b>
732-0083	<b>Econo-Pac CHT-II Cartridge, 5 x 1 ml</b>	Ceramic hydroxyapatite
<b>Other Econo-Pac cartridges</b>		
732-0081	<b>Econo-Pac CHT-II Cartridge, 1 x 5 ml</b>	
732-0085	<b>Econo-Pac CHT-II Cartridge, 5 x 5 ml</b>	
All 1 ml Econo-Pac cartridges are also available in a 5 ml cartridge format.		
Larger package sizes for media are available for process scale chromatography.		
Inquire with your local Bio-Rad representative.		
<b>Fittings Kits</b>		
732-0111	<b>Econo-Pac Cartridge - FPLC Adaptor Fittings Kit</b>	
732-0112	<b>Econo-Pac Cartridge - HPLC Adaptor Fittings Kit</b>	

\* US Patent 4,871,463

FPLC is a registered trademark of Pharmacia.

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

**LIT533 Rev C**