Econo-Pac® Ion Exchange Cartridges

Instruction Manual

Catalog Numbers
732-0023
732-0063
732-0003

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, ion exchange,hydroxyapatite, 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 Q, S, and CM cartridges are packed with Macro-Prep® ion exchange supports. These supports are based on a spherical, rigid polymer with a narrow particle size distribution, which allows improved resolution and high flow rates. These ion exchange cartridges are used for the research scale fractionation of complex mixtures of biomolecules. Detailed product information is given in Table 1.

Section 2
Connecting to Bio-Rad's Econo System

The Econo-Pac cartridges are ideal for use with Bio-Rad's Econo System, a low pressure chromatography system. The Econo-Pac ion exchange cartridges 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.

Table 1. Description of Econo-Pac Ion Exchange Cartridges

	Econo-Pac Q cartridge	Econo-Pac S cartridge	Econo-Pac CM cartridge
Type	Strongly basic anion exchanger	Strongly acidic cation exchanger	Weakly acidic cation exchanger
Functional group	$-N^+(CH_3)_3$	$-SO_3^-$	$-COO^-$
Counterion	Chloride	Sodium	Sodium
Bed volume	1 ml	1 ml	1 ml
Protein binding capacity	≥ 15 mg ferritin	≥ 35 mg human IgG	≥ 25 mg hemoglobin
Ionic capacity (matrix)	190 ± 40 µeq	160 ± 40 µeq	210 ± 40 µeq
Particle diameter (nominal)	50 µm	50 µm	50 µm
Pore size (nominal)	1,000 Å	1,000 Å	1,000 Å
Recommended flow rate	0.5-1.0 ml/min	0.5-1.0 ml/min	0.5-1.0 ml/min
Maximum flow rate	6 ml/min	6 ml/min	6 ml/min
Operating pH range	2-12	2-12	2-12
Average back pressure	11 psi at 6 ml/min (water at 20 °C)		

	Econo-Pac Q cartridge	Econo-Pac S cartridge	Econo-Pac CM cartridge
Maximum operating pressure**	3.4 bar (50 psi) at 20 °C.		
Cartridge and frit construction	Polypropylene	Polypropylene	Polypropylene
Shipping conditions	Semi-dry	Semi-dry	Semi-dry
Recommended storage	20 mM phosphate, pH 6.8,with 0.05% NaN_3; or 20% v/v ethanol solution		

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 ⅛ 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

Econo-Pac ion exchange cartridges are packed using sterile buffer and are 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. Equilibrate 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 that to be used in 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, the sample should be exchanged into the starting buffer or diluted to the starting buffer's concentration.

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. 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 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

4.2 General Purification Protocol

Ion exchange chromatography is usually performed using increasing salt gradients or pH 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 cation and anion exchange chromatography are listed in Table 3.

An appropriate starting point for separation of many samples is a linear gradient from 0 to 1.0 M NaCl over 25 minutes at a flow rate of 0.7 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 6 ml/min with 10-20 ml of buffer containing 1.0 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 of the same type can be connected in series. Econo-Pac cartridges are available in a 5 ml cartridge format. The Macro-Prep ion exchange supports are also available in larger amounts, from 100 ml to bulk quantities, for scaling up methods developed using the cartridges. In addition, Bio-Rad carries an extensive line of empty chromatography columns.

Table 3. Common Buffers for Ion Exchange Chromatography[1,2,3]

Type of Ion Exchanger	Buffer	Buffering Range
Cation	Acetic acid	4.8-5.2
	Citric acid	4.2-5.2
	HEPES	7.6-8.2
	Lactic Acid	3.6-4.3
	MES	5.5-6.7
	MOPS	6.5-7.9
	Phosphate	6.7-7.6
	PIPES	6.1-7.5
	Pivalic acid	4.7-5.4
	TES	7.2-7.8
	Tricine	7.8-8.9
Anion	Bicine	7.6-9.0
	Bis-Tris	5.8-7.2
	Diethanolamine	8.4-8.8
	Diethylamine	9.5-11.5
	L-Histidine	5.5-6.0
	Imidazole	6.6-7.1
	Pyridine	4.9-5.6
	Tricine	7.8-8.9
	Triethanolamine	7.3-8.0
	Tris	7.5-8.0

Section 5
Care of the Cartridge

5.1 Cleaning the Cartridge

After repeated use, an ion exchange 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 20-30 ml of 1 M NaOH at 1 ml/min.
2. Wash with 50 ml of deionized water or starting buffer (6 ml/min).
3. Wash with 25 ml of high salt buffer (6 ml/min).
4. Equilibrate the cartridge with at least 25 ml of starting buffer (6 ml/min).

If bound contaminants persist after following the procedure above, use one of both of the alternative procedures below:

Wash Alternative 1

Perform step 2 above. Wash with 50 ml of 20% ethanol solution. Then continue with steps 2-4 above.

Wash Alternative 2

Perform step 2 above. Wash with 25 ml of 75% acetic acid or 1.0 M HCl. Then continue with steps 2-4 above.

5.2 Autoclaving

Econo-Pac ion exchange 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. Autoclaved cartridges should be prepared as described in the Preparing a Cartridge For Use section of this manual.

5.3 Storage

The Econo-Pac ion exchange 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
	Econo-Pac Ion Exchange 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
	Other Econo-Pac Cartridges	
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
732-0093	**Econo-Pac Protein A Cartridge, 5 x 1 ml**	Affinity

All 1 ml Econo-Pac cartridges are also available in a 5 ml cartridge format. Larger package sizes of media are available for process scale chromatography. Inquire with your local Bio-Rad representative.

* US Patent 4,871,463

** Pressure unit is for the cartridge. The Macro-Prep supports are stable to pressures up to 68 bar (1000 psi).

Section 8 References

1. Harris, E. L. V. and Angal, S., **Protein Purification Methods, A Practical Approach,** IRL Press, Oxford, 1989.

2. Scopes, R. K., **Protein Purification, Principles and Practice.** (Second Edition), Springer-Verlag, New York, 1987.

3. Snyder, L. R. and Kirkland, J. J., **Introduction to Modern Liquid Chromatography** (Second Edition), John Wiley & Sons, Inc., New York, 1979.

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