



Econo-Pac[®] DEAE Blue and Econo-Pac Blue Cartridges

Catalog Numbers

732-0031

732-0035

732-0101

732-0105

BIO-RAD

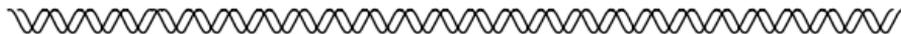


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Section 1

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, affinity, hydroxylapatite and hydrophobic interaction. See Ordering Information for a listing of the 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.

DEAE Affi-Gel® blue and Affi-Gel blue dye affinity supports are based on a spherical, crosslinked agarose matrix with a narrow particle size distribution, which allows excellent resolution and moderate flow rates.

The Econo-Pac DEAE blue cartridge is packed with DEAE Affi-Gel blue gel. The gel combines the properties of dye affinity and anion exchange to provide a unique selectivity for IgG-type antibodies. This cartridge is used to purify IgG from both serum or ascites samples. Request bulletin 1092 for information on other applications.

The Econo-Pac blue cartridge is packed with Affi-Gel blue gel, a dye affinity support for albumin removal and for the purification of NAD and NADP-dependent enzymes or blood proteins. Request bulletin 1107 for information about other applications. Detailed product technical data and chromatographic characteristics are given in Table 1.

Table 1. Description of Econo-Pac Dye Affinity Cartridges

	Econo-Pac DEAE Blue Cartridge	Econo-Pac Blue Cartridge
Type	Dye affinity, weakly basic anion exchanger	Dye affinity
Functional group(s)	Cibacron® Blue F3GA and (DEAE)-O(CH ₂) ₂ N(CH ₂ CH ₂) ₂ HCl	Cibacron Blue F3GA
Bed volume	5 ml	5 ml
Serum capacity	0.3 - 1.0 ml	0.3 - 1.0 ml
Particle diameter (nominal)	120 µm	120 µm
Recommended flow rate	0.5-2.0 ml/min	0.5 - 2.0 ml/min
Maximum flow rate	3.0 ml/min	3.0 ml/min
Operating pH range	2 - 10	2 - 10
Average back pressure	136 mbar (2 psi) at 3.0 ml/min (low ionic strength buffer at 20° C)	
Maximum operating pressure*	680 mbar (10 psi or .36 mPa) at 20 °C	680 mbar (10 psi or .36 mPa) at 20 °C
Cartridge and frit construction	Polypropylene	Polypropylene
Shipping conditions	Fully hydrated in 0.020 M sodium phosphate buffer, pH 8.0, 0.01% NaN ₃	

(continued on next page)

Table 1. (continued)

Recommended storage	0.020 M sodium phosphate buffer, pH 8.0, with 0.01% NaN ₃ , at 4 °C
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* Although the pressure limitation for the cartridge is 3.4 bar (50 psi) at 20 °C, these agarose supports are limited to lower pressures, approximately 680 mbar (10 psi).

Section 2

Connecting to Bio-Rad's Econo System

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

Caution: Do not run the purge mode with the Econo-Pac DEAE blue or Econo-Pac blue cartridges. 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. See Table 1 for the maximum flow rate for these Econo-Pac cartridges.

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 and pressure limit for the cartridges are not exceeded. Higher pressures are usually caused by restrictions in tubing or detector cells downstream from the cartridge. If possible, set the pressure limit of the system according to the maximum operating pressure of 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. 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

Preparing a Cartridge For Use

Both Econo-Pac affinity cartridges are packed with sterile buffer containing sodium azide and are shipped in a fully hydrated state to maximize shelf life. New cartridges should always be prepared according to the instructions in this section. Refer to Table 2 for the formulation of the buffers discussed in this section.

If air is accidentally introduced to a cartridge, it can be easily removed following these same instructions. After connecting the cartridge to a liquid chromatography system, prepare it as follows:

1. Set pump flow rate to 1.0 ml/min.
2. Wash the cartridge with degassed regeneration buffer (G) for 10 minutes at 1.0 ml/min. This wash will elute any residual dye which might otherwise be eluted during chromatography.
3. Wash the cartridge with degassed elution buffer (D or E) for 10 minutes at 2.0 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 degassed application buffer (A, B, or C) for 10 minutes at 2.0 ml/min.
5. Equilibrate the cartridge with degassed application buffer for 2 minutes at 1.0 ml/min.
6. Invert the cartridge so that the cartridge points downward.

Table 2. Buffer Formulations

Application buffers

- A. 0.028 M NaCl, 0.020 M Tris-HCl, pH 8.0
- B. 0.020 M K_2HPO_4 , pH 8.0
- C. 0.020 Na_2HPO_4 , pH 7.1

Elution buffers

- D. 0.4 M K_2HPO_4 , pH 8.0
- E. 1.4 M NaCl, 0.020 M Tris-HCl, pH 8.0
- F. 1.4 M NaCl, 0.020 M Na_2HPO_4 , pH 7.1

Regeneration buffers

- G. 1.4 M NaCl, 0.10 M acetic acid, pH 3.0, 40% v/v isopropanol
 - H. 1.5 M sodium thiocyanate in application buffer A, B, or C
 - I. 2.0 M guanidine-HCl in application buffer A, B, or C
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Table 3. Recommended Application Buffer for Specific Samples

Sample	Target Compound	Econo-Pac Cartridge	Buffer
Rabbit, rat, goat, or sheep serum	IgG	DEAE Blue	A
Human serum	IgG	DEAE Blue	B
Serum	Albumin	Blue	C
Physiological fluids	Enzymes	Blue	Varies

4.1 Sample Preparation

Proper adjustment of the sample pH and ionic strength is critical for consistent and reproducible results when using Econo-Pac dye affinity cartridges. The sample must be exchanged into the appropriate application buffer. This can be achieved by exchanging it into the application buffer using the Econo-Pac P6 cartridge, Econo-Pac 10DG desalting columns, or Bio-Gel® P-6DG gel filtration gel. The choice of product depends on the sample volume. Alternatively, the sample can be dialyzed against the application buffer. All samples should be filtered through a 0.45 µm filter.

4.2 General Purification Protocol

Isocratic or increasing salt gradient conditions are often used to fractionate samples on the Econo-Pac dye affinity cartridges. Table 3 references selected buffers for some specific applications. Buffer formulations are given in Table 2.

4.3 Scaling Up the Separation

Larger quantities of the dye affinity supports and an assortment of empty chromatography columns are available for scaling up applications developed using the cartridges.

Section 5 Specific Purification Protocols

Several specific application protocols have been developed using dye affinity supports. Examples follow.

5.1 Purification of IgG from Serum or Ascites with the Econo-Pac DEAE Blue Cartridge

IgG can be isolated from serum or ascites samples using the Econo-Pac DEAE blue cartridge. The resulting purified IgG fraction may contain a residual amount of transferrin.

1. Equilibrate the cartridge in application buffer (A or B).
2. Apply the prepared sample to the cartridge.
3. Elute the IgG with 10-20 ml application buffer. Smaller volume fractions should be collected for more precise collection of the IgG fraction.
4. Optional: Most of the bound albumin can be eluted by washing the cartridge with 10-20 ml of elution buffer (D or E).
5. Regenerate the cartridge as recommended in the Cleaning the Cartridge section.

5.2 Purification of Serum Proteins with the Econo-Pac DEAE Blue Cartridge

Serum proteins have been purified using linear gradients on DEAE Affi-Gel blue gel.¹ For gradient separations on the Econo-Pac DEAE blue cartridge, an appropriate starting point is a linear gradient from application buffer (B) to Elution buffer (D) over 60 minutes. The remaining bound proteins are eluted with elution buffer (E). The flow rate is usually set between 0.5 and 2.0 ml/min. The separation can then be optimized by changing the flow rate and gradient profile. Request Technical bulletin 1092 for further information of the purification of serum proteins using DEAE Affi-Gel blue gel.

5.3 Removal of Albumin from Serum with the Econo-Pac Blue Cartridge

The Econo-Pac blue cartridge can provide a simple first step in the purification of many serum proteins by removing the major serum component, albumin.

1. Equilibrate the cartridge in application buffer (C).
2. Apply the prepared sample to the cartridge.

3. Wash the cartridge with 10-15 ml of application buffer (C). The effluent from this step contains the serum proteins minus most of the albumin.
4. Optional: Most of the bound albumin can be eluted by washing the cartridge with 10-20 ml of elution buffer (F).
5. Regenerate the cartridge as recommended in the Cleaning the Cartridge section.

5.4 Purification of Enzymes with the Econo-Pac Blue Cartridge

The Econo-Pac blue cartridge can be used to purify a number of enzymes, especially kinases, dehydrogenases, and other nucleotide-dependent enzymes.

1. Equilibrate the cartridge in application buffer. The application buffer will vary depending upon the enzyme to be purified. In general, the application buffer should be low ionic strength, 0.05 M or less, with pH between 6.0-8.5.
2. Apply the prepared sample to the cartridge.
3. Wash the cartridge with 10 ml application buffer.

4. Check the effluent for enzyme activity. If the enzyme of interest is bound by the cartridge, proceed to step 5. If the enzyme is not bound, alter the application conditions: change the pH, change the buffer, or decrease ionic strength.
5. The enzyme can be eluted with a salt gradient² or with a competitive eluant such as a cofactor. Examples of salt gradients include 0.05 to 1.5 M NaCl^{3, 4, 5} or 0 to 3.0 M KCl.^{6, 7, 8} Request technical bulletin 1107 for more information on elution buffers for enzyme purification.
6. Regenerate the cartridge as recommended in the Cleaning the Cartridge section.

Section 6 Care of the Cartridge

6.1 Cleaning the Cartridge

After each use, both Econo-Pac dye affinity cartridges require thorough cleaning and regeneration to remove bound contaminants. Referring to Table 3 for

buffers, bound contaminants may be removed by following the procedure below:

1. Set the pump flow rate to 2.0 ml/min.
2. Wash the cartridge with 10 ml of elution buffer (E or F).
3. Wash with 10 ml regeneration buffer (H or I).
4. Wash with 20 ml application buffer (A, B, or C).
5. Reduce the flow to 1.0 ml/min.
6. Continue with sample application.

6.2 Storage

The Econo-Pac dye affinity cartridges should be stored at 4 °C in 0.020 M sodium phosphate buffer containing 0.02% sodium azide. Perform steps 1-3 in the Cleaning the Cartridge section, then wash with storage buffer.

6.3 Autoclaving

The Econo-Pac DEAE blue and Econo-Pac blue cartridges are not autoclavable.

Section 7 Technical Assistance

For additional information and technical assistance, contact your local Bio-Rad representative or, in the United States, call Technical Service at 1-800-4BIORAD.

Section 8 Ordering Information

Catalog Number	Product Description	Type
<i>Econo-Pac dye affinity cartridges</i>		
732-0031	Econo-Pac DEAE Blue Cartridge, 1x5 ml	Dye affinity, weakly basic anion exchanger
732-0035	Econo-Pac DEAE Blue Cartridge, 5x5 ml	Dye affinity, weakly basic anion exchanger
732-0101	Econo-Pac Blue Cartridge, 1x5 ml	Dye affinity
732-0105	Econo-Pac Blue Cartridge, 5x5 ml	Dye affinity

Catalog Number	Product Description	Type
Other Econo-Pac cartridges		
732-0001	Econo-Pac CM Cartridge, 1x5 ml	Weakly acidic cation exchanger
732-0005	Econo-Pac CM Cartridge, 5x5 ml	Weakly acidic cation exchanger
732-0061	Econo-Pac S Cartridge, 1x5 ml	Strongly acidic cation exchanger
732-0065	Econo-Pac S Cartridge, 5x5 ml	Strongly acidic cation exchanger
732-0021	Econo-Pac Q Cartridge, 1x5 ml	Strongly basic anion exchanger
732-0025	Econo-Pac Q Cartridge 5x5 ml	Strongly basic anion exchanger
732-0051	Econo-Pac Methyl HIC Cartridge 1x5 ml	Hydrophobic interaction
732-0055	Econo-Pac Methyl HIC Cartridge, 5x5 ml	Hydrophobic interaction
732-0071	Econo-Pac Heparin Cartridge, 1x5 ml	Affinity
732-0075	Econo-Pac Heparin Cartridge, 5x5 ml	Affinity
732-0011	Econo-Pac P6 Cartridge, 1x5 ml	Desalting

Catalog Number	Product Description	Type
732-0015	Econo-Pac P6 Cartridge, 5x5 ml	Desalting
732-0081	Econo-Pac HTP Cartridge, 1x5 ml	Hydroxylapatite
732-0085	Econo-Pac HTP Cartridge, 5x5 ml	Hydroxylapatite
732-0091	Econo-Pac protein A Cartridge, 1x5 ml	Affinity
Fittings		
732-0111	Econo-Pac Cartridge-FPLC adaptor fittings kit	
732-0112	Econo-Pac Cartridge-HPLC adaptor fittings kit	
For scale up		
153-7307	DEAE Affi-Gel Blue Gel, 100 ml	
153-7302	Affi-Gel Blue Gel, 100 ml	

FPLC is a registered trademark of Pharmacia. Cibacron is a registered trademark of Ciba-Geigy.

*U.S. Patent 4,871,463

Section 9

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