EconoFit Affi-Gel Blue and DEAE Affi-Gel Blue Columns, 5 ml

Instruction Manual

Catalog number

12009234
12009235
12009262
12009263

Please read the instructions in this manual prior to using EconoFit Affi-Gel Blue and DEAE Affi-Gel Blue Columns. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Section 1
Introduction

EconoFit Affi-Gel Blue and DEAE Affi-Gel Blue Columns are convenient, disposable, prepacked low-pressure chromatography columns. EconoFit Columns offer both increased run-to-run uniformity and high purity of protein through the column design and novel resin technology. Compatible with aqueous buffers most commonly used for protein purification, EconoFit Columns offer improved performance for your protein separation needs.

These columns are packed with Affi-Gel Affinity Chromatography Gels. These gels are hydrophilic, spherical, crosslinked agarose beads designed for the purification of proteins or the removal of high-abundance proteins from cell lysates or serum. Download bulletin 1107 from **bio-rad.com** for other applications. Affi-Gel Beads are designed to provide medium capacity, low backpressure, and high productivity.

Section 2
Product Information

EconoFit Columns are supplied ready for use in convenient 1 and 5 ml sizes. They can be quickly connected to liquid chromatography systems using 10-32 fittings. Columns are available for a variety of chromatographic techniques, including desalting (size exclusion [SEC]), ion exchange (IEX), affinity (AC), mixed-mode, and hydrophobic interaction chromatography (HIC). See Table 1 for specifications. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of items in the EconoFit Column product line.

Table 1. EconoFit Affi-Gel Blue and DEAE Affi-Gel Blue Column specifications.

Property	Description
Size	5 ml bed volume
Bed dimensions	1 ml: 25 mm length x 7 mm inner diameter 5 ml: 25 mm length x 16 mm inner diameter
Maximum pressure tolerance	10 psi (680 mbar/0.36 mPa)*
Recommended flow rate	0.5–2.0 ml/min (25–100 cm/hr)
Fittings	10-32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Shipping solution	0.02 M sodium phosphate, pH 7.4, 0.05% NaN_3
Storage conditions	0.02 M sodium phosphate, pH 7.4, 0.05% NaN_3
Autoclavability	Not autoclavable

* Although the pressure limitation of the column is 72 psi, the agarose gel tends to compress above 10 psi (generally 3.0 ml/min).

Affi-Gel Blue and DEAE Affi-Gel Blue Resins are also available in bottles. Refer to Ordering Information in section 7 of this manual. See Table 2 for specifications. Go to **bio-rad.com/ResinsandColumns** for more information.

Table 2. Affi-Gel Blue and DEAE Affi-Gel Blue Resin specifications.

Property	Affi-Gel Blue Description	DEAE Affi-Gel Blue Description
Type	Dye affinity	Dye affinity/weak anion
Functional group	Cibacron Blue F3GA	Cibacron Blue F3GA and $-N(C_2H_5)_2$
Serum capacity	0.3–1.0 ml	0.3–1.0 ml
Maximum flow rate	3.0 ml/min	3.0 ml/min
Operating pH range	2–10	2–10
pH stability	1–10	1–10

Section 3
Preparing a Column and Subsequent Purification

EconoFit Affi-Gel Blue and DEAE Affi-Gel Blue Columns contain 0.02 M sodium phosphate buffer, pH 7.4, and 0.05% sodium azide as the storage solution. The fully hydrated support is ready to use after equilibrating the column in the buffer of choice. To perform buffer exchange, connect the column to a liquid chromatography system or peristaltic pump and condition it as instructed.

If air is accidently introduced to a column, it can be easily removed following these same instructions. After connecting the column to a liquid chromatography system, prepare it as follows, using Table 3 as a guide.

1. Set pump flow rate to 1.0 ml/min.
2. Wash the column with degassed regeneration buffer (G) for 10 min at 1.0 ml/min.
3. Wash the column with degassed elution buffer (D or E) for 10 min at 2.0 ml/min.
4. Wash the column with degassed application buffer (A, B, or C) for 10 min at 2.0 ml/min.
5. Equilibrate the column with degassed application buffer for 2 min at 1.0 ml/min.

Table 3. Buffer formulations.

Buffer	Formulation
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 M 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 isopropyl alcohol
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

Sample Preparation

Proper pH and ionic strength are necessary for consistent and reproducible results. Sample can be exchanged into the starting buffer or diluted to the starting buffer 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 EconoFit Bio-Gel P6 Desalting Columns, Micro Bio-Spin P-6 or Micro Bio-Spin P-30 Columns, Bio-Spin P-6 or Bio-Spin P-30 Columns, Econo-Pac 10DG Desalting Columns, or Bio-Gel P-6DG Gel, as listed in Table 4. The choice of product will depend on the sample volume. All samples should be filtered through a 0.45 µm filter prior to column application.

Table 4. Products for buffer exchange.

Sample Volume	Recommended Product	Use	Catalog #
10–75 µl	Micro Bio-Spin P-6 Column	Desalting proteins over 6 kD	7326221
10–75 µl	Micro Bio-Spin P-30 Column	Desalting proteins over 30 kD	7326223
50–100 µl	Bio-Spin P-6 Column	Desalting proteins over 6 kD	7326227
50–100 µl	Bio-Spin P-30 Column	Desalting proteins over 30 kD	7326231
100 µl–3 ml	EconoFit Bio-Gel P6 Desalting Column	Desalting proteins over 6 kD	12009239
Up to 3 ml	Econo-Pac 10DG Desalting Columns	Desalting proteins over 6 kD	7322010
Unlimited	Bio-Gel P-6DG Gel	Desalting proteins over 6 kD	1500738

General Purification Protocol

Affinity chromatography can be performed using isocratic elution or by increasing salt or pH gradients to fractionate the sample components. For best results and increased column life, samples and buffers should be degassed and filtered through a 0.45 µm filter. Table 5 references selected buffers for some specific applications. Buffer formulations are listed in Table 3.

Table 5. Recommended application buffer for specific samples.

Sample	Target Compound	EconoFit Column	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

Section 4
Scaling Up

For quick scale-up, two or three columns of the same type can be connected in series. Backpressure will increase with columns in series, so care should be taken to maintain pressures ≤10 psi. EconoFit Affi-Gel Blue and DEAE Affi-Gel Blue Columns are available in a 5 ml format. The Affi-Gel Blue and DEAE Affi-Gel Blue Resins are also available in 100 ml bottles for scaling up methods developed using the columns.

In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory to process scale. Ask your local Bio-Rad representative or go to **bio-rad.com/ResinsandColumns** for more information.

Section 5
Specific Purification Protocols

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

Purification of IgG from Serum or Ascites with the EconoFit DEAE Affi-Gel Blue Column

IgG can be isolated from serum or ascite samples using the EconoFit DEAE Affi-Gel Blue Column. The resulting purified IgG fraction may contain a residual amount of transferrin.

1. Equilibrate the column in application buffer (A or B).
2. Apply the prepared sample to the column.
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 column with 10–20 ml of elution buffer (D or E).
5. Regenerate the column as recommended in Cleaning the Column (in section 6).

Purification of Serum Proteins with the EconoFit DEAE Affi-Gel Blue Column

Serum proteins have been purified using linear gradients on DEAE Affi-Gel Blue Gel (Williams 1967). For gradient separations on the EconoFit DEAE Blue Column, 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.

Removal of Albumin from Serum with the EconoFit Affi-Gel Blue Column

The EconoFit Affi-Gel Blue Column can provide a simple first step in the purification of many serum proteins by removing the major serum component, albumin.

1. Equilibrate the column in application buffer (C).
2. Apply the prepared sample to the column.
3. Wash the column 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 column with 10–20 ml of elution buffer (F).
5. Regenerate the column as recommended in Cleaning the Column (in section 6).

Purification of Enzymes with the EconoFit Affi-Gel Blue Column

The EconoFit Affi-Gel Blue Column can be used to purify a number of enzymes, especially kinases, dehydrogenases, and other nucleotide-dependent enzymes.

1. Equilibrate the column 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 and 8.5.
2. Apply the prepared sample to the column.
3. Wash the column with 10 ml application buffer.
4. Check the effluent for enzyme activity. If the enzyme of interest is bound by the column, 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 gradient (Wilson 1976) or with a competitive eluent such as a cofactor. Examples of salt gradients include 0.05–1.5 M NaCl (Keith et al. 1982, Sharma et al. 1980, Tomasselli and Noda 1980) or 0.0–3.0 M KCl (Bisson and Thorner 1981, Chetsanga et al. 1981, Kattchee 1981). Download bulletin 1107 from **bio-rad.com** for more information on elution buffers for enzyme purification.
6. Regenerate the column as recommended in Cleaning the Column (in section 6).

Section 6
Care of the Column

Cleaning the Column

After each use, both types of EconoFit Dye Affinity Columns require thorough cleaning and regeneration to remove bound contaminants. Referring to Table 3 for buffers, remove contaminants by following this procedure:

1. Set the pump flow rate to 2.0 ml/min.
2. Wash the column 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.

Storage

EconoFit Dye Affinity Columns should be stored at 4°C in 0.020 M sodium phosphate buffer containing 0.05% sodium azide. Perform steps 1–3 in the Cleaning the Column section, then wash with storage buffer.

Section 7
Ordering Information

Catalog #	Description
EconoFit Affi-Gel Blue Columns	
12009234	**EconoFit Affi-Gel Blue Column**, 1 x 5 ml column
12009235	**EconoFit Affi-Gel Blue Columns**, 5 x 5 ml columns
EconoFit DEAE Affi-Gel Blue Columns	
12009262	**EconoFit DEAE Affi-Gel Blue Column**, 1 x 5 ml column
12009263	**EconoFit DEAE Affi-Gel Blue Columns**, 5 x 5 ml columns
Affi-Gel Blue Resins	
1537301	**Affi-Gel Blue Resin, 50–100 mesh**, 100 ml bottle
1537302	**Affi-Gel Blue Resin, 100–200 mesh**, 100 ml bottle
DEAE Affi-Gel Blue Resin	
1537307	**DEAE Affi-Gel Blue Resin**, 100 ml bottle

Section 8
References

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Keith JM et al. (1982). Purification and characterization of the messenger ribonucleic acid capping enzyme GTP:RNA guanylyltransferase from wheat germ. Biochemistry 21, 327–333.

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Williams CA (1967). Methods in Immunology and Immunochemistry, C.A. Williams and M.W. Chase, eds. (New York: Academic Press).

Wilson JE (1976). Applications of blue dextran and Cibacron Blue F3GA in purification and structural studies of nucleotide-requiring enzymes. Biochem Biophys Res Commun 72, 816–823.

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