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Bio-Scale™ Mini Affi-Gel®  
Blue and DEAE Affi-Gel  
Blue Cartridges, 5 ml

Instruction Manual

Catalog #

732-4642, 732-4644

732-4632, 732-4634

**BIO-RAD**



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# Section 1

## Introduction

Bio-Scale Mini cartridges have a patent-pending double-wall design that provides extra durability and allows easy, reliable runs with aqueous buffers commonly used for protein purification. The polypropylene luer fittings and internal sealing surfaces ensure leak-free operation at pressures up to 45 psi. Bio-Scale Mini cartridges are convenient, disposable, and supplied ready for use. They are easy to use and prepacked for fast, reproducible chromatographic separations. Cartridges are available for a variety of chromatographic techniques, including desalting, ion exchange, affinity, and mixed-mode chromatography. The design of Bio-Scale Mini cartridges offers:

- Ready-to-use convenience; simply equilibrate the cartridge in the buffer of choice
- Luer fittings for convenient connection to any chromatography system or directly to a Luer-Lok syringe

Bio-Scale Mini Affi-Gel Blue and DEAE Affi-Gel Blue cartridges 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. Request bulletin 1107 for other applications. Affi-Gel beads are designed to provide medium capacity, low backpressure, and high productivity. Detailed product information is given in Tables 1 and 2. See Ordering Information for a listing of the complete Bio-Scale Mini cartridge product line.



## **Table 1. Bio-Scale Mini Affi-Gel Blue and DEAE Affi-Gel Blue cartridge specifications.**

Size	5 ml bed volume
Dimensions	40 mm length x 12.6 mm inner diameter
Maximum pressure tolerance	10 psi (680 mbar. 0.36 mPa)*
Operational flow rates	0.5–2.0 ml/min (25–100 cm/hr)
Fittings:	Female luer fitting inlet; male luer fitting outlet
Column material	Polypropylene
Frit material	Polyethylene (HDPE)
Shipping conditions	0.02 M sodium phosphate, pH 7.4, 0.05% NaN <sub>3</sub>
Storage recommendations	0.02 M sodium phosphate, pH 7.4, 0.05% NaN <sub>3</sub>
Autoclavability	Not autoclavable

\*Although the pressure limitation of the cartridge is 45 psi, the agarose gel tends to compress above 10 psi (generally 3.0 ml/min).

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

	<b>Affi-Gel Blue</b>	<b>DEAE Affi-Gel Blue</b>
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	1–12
Chemical compatibility	See Table 3.	

# Section 2

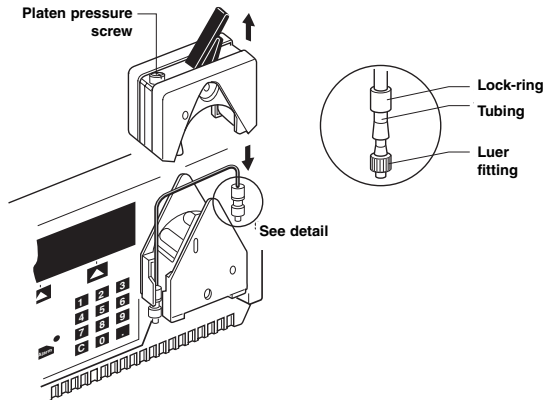
## Connecting to Bio-Rad's Low-Pressure Chromatography Instruments

Bio-Scale Mini cartridges are ideal for use with Bio-Rad's BioLogic™ LP chromatography system, Econo™ gradient pump, the patented\* Model EP-1 Econo™ pump, and all low-pressure chromatography instruments. Bio-Scale Mini cartridges can be conveniently connected directly to the system using the luer fittings on the cartridge.

1. Install 1.6 mm inner diameter (ID) tubing in the pumphead. Adjust the platen pressure screw (on pumphead) – using a screwdriver or coin, turn the screw counterclockwise as far as it will go, then turn clockwise three full turns. Assemble with fittings and lock rings as shown in Figure 1.

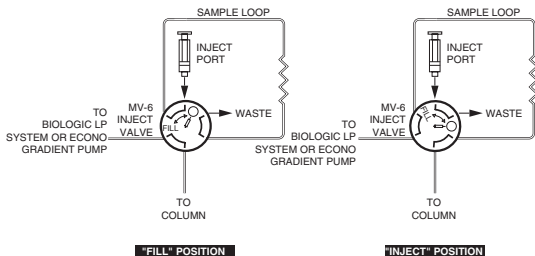
\*US Patent 5,135,658

(Use orange lock rings and medium size barb fittings with 1.6 mm tubing.)



**Fig. 1. Biologic LP system setup.**

2. To maximize gradient accuracy and to apply samples efficiently, install 1.6 mm ID tubing from the pump to the MV-6 sample inject valve (if available). If using the MV-6 sample inject valve, turn the knob counterclockwise as far as it will go so it will now correspond to the printed diagram on the valve (see Figure 2).



**Fig. 2. Connecting to an MV-6 valve.**

3. Connect the inlet of the cartridge to the male luer fitting on the MV-6 sample inject valve (see Figure 2). If not using the MV-6 sample inject valve, connect a barb to male luer fitting on the 1.6 mm ID tubing, then connect to the top of the female luer on the Bio-Scale Mini cartridge. For optimum performance, a cartridge should be mounted vertically with the arrow on the cartridge pointing downward (see Figure 3).
4. Connect the cartridge outlet to the 1.6 mm ID tubing leading to the BioLogic LP system optics module or to the Model EM-1 Econo™ UV monitor. It is recommended to use the shortest length (approximately 10 cm) of 1.6 mm ID tubing. Connect a barb to female luer to the 1.6 mm ID tubing, then connect to the bottom of the male luer on the Bio-Scale Mini cartridge.



**Fig. 3. Cartridge and fittings.**

# Section 3

## Connecting to Other Liquid Chromatography Systems

Bio-Scale Mini cartridges can be connected to any liquid chromatography system, provided that the maximum pressure limit (3 bar, 45 psi, or 300 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 fitting kits for easy connection of a Bio-Scale Mini cartridge to a BioLogic DuoFlow, HPLC, or FPLC-type system.

### **3.1 BioLogic DuoFlow Systems**

The luer to BioLogic system fittings kit (catalog #732-0113) includes 1/4-28 female to male luer and 1/4-28 female to female luer to connect one Bio-Scale Mini cartridge to the BioLogic DuoFlow system, see Figure 4.





**Fig. 4. Luer to 1/4-28 adaptor.**

### **3.2 HPLC Systems**

The luer to 10-32 adaptor fittings kit (catalog #732-0112) provides fittings necessary to connect the Bio-Scale Mini 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 the 1/16 inch OD stainless-steel tubing to a distance of 1 cm.

### **3.3 FPLC Systems**

The luer to M6 adaptor fittings kit (catalog #732-0111) provides fittings necessary to connect the Bio-Scale Mini cartridge to the M6 fittings found on FPLC or related systems. Alternatively, connection can be made by using one GE Healthcare Union luer lock female to M6 female fitting (GE 18-1027-12) and one female luer lock to M6 male fitting (Upchurch P-686 or GE 18-1027-62). To prevent tubing or cartridge failure, do not exceed the maximum recommended flow rate of the cartridge.

# Section 4

## Preparing a Cartridge For Use

Bio-Scale Mini Affi-Gel Blue and DEAE Affi-Gel Blue cartridges 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 cartridge in the buffer of choice. To perform buffer exchange, connect the cartridge to a liquid chromatography system or peristaltic pump and condition it as instructed below:

Both Bio-Scale Mini 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 3 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 using Table 3 as a guide.

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.
3. Wash the cartridge with degassed elution buffer (D or E) for 10 minutes at 2.0 ml/min.
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.

## **Table 3. 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 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

**Table 4. Recommended application buffer for specific samples.**

<b>Sample</b>	<b>Target Compound</b>	<b>Bio-Scale Mini Cartridge</b>	<b>Buffer</b>
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 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'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 a Bio-Scale Mini P-6 cartridge, Bio-Spin<sup>®</sup> 6 or Bio-Spin 30 column, Econo-Pac<sup>®</sup> 10DG desalting column, or Bio-Gel<sup>®</sup> P-6 DG gel filtration gel (see Table 5). The choice of product will depend on sample volume. All

samples should be filtered through a 0.45  $\mu\text{m}$  filter prior to cartridge application.

**Table 5. Products for buffer exchange.**

<b>Sample Volume</b>	<b>Recommended Product</b>	<b>Use</b>	<b>Catalog #</b>
50–100 $\mu\text{l}$	Bio-Spin 6 column	Desalting proteins $\geq 6$ kD	732-6002
50–100 $\mu\text{l}$	Bio-Spin 30 column	Desalting proteins $\geq 30$ kD	732-6006
100 $\mu\text{l}$ –3 ml	Bio-Scale Mini P-6 cartridge	Desalting proteins $\geq 6$ kD	732-4502
Up to 3 ml	Econo-Pac 10DG desalting column	Desalting proteins $\geq 6$ kD	732-2010
Unlimited	Bio-Gel P-6DG gel	Desalting proteins $\geq 6$ kD	150-0738

## **4.2 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 cartridge life, samples, and buffers should be degassed and filtered through a 0.45  $\mu\text{m}$  filter. Table 4 references selected buffers for some specific applications. Buffer formulations are listed in Table 3.

## **4.3 Scaling Up the Separation**

For quick scale-up, two or three cartridges of the same type can be connected in series. Backpressure will increase with cartridges in series, so care should be taken to maintain pressures  $\leq 10$  psi. Bio-Scale Mini Affi-Gel Blue and DEAE Affi-Gel Blue cartridges are available in 5 ml format. The Affi-Gel Blue and DEAE Affi-Gel Blue media are also available in 100 ml bottles for scaling up methods developed using the cartridges. In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory scale to process scale.



# 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 Bio-Scale Mini DEAE Affi-Gel Blue Cartridge**

IgG can be isolated from serum or ascite samples using the Bio-Scale Mini DEAE Affi-Gel 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 Section 6.1, Cleaning the Cartridge.

## **5.2 Purification of Serum Proteins With the Bio-Scale Mini DEAE Affi-Gel Blue Cartridge**

Serum proteins have been purified using linear gradients on DEAE Affi-Gel Blue gel.<sup>1</sup> For gradient separations on the Bio-Scale Mini 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 bulletin 1092 for further information on the purification of serum proteins using DEAE Affi-Gel blue gel.

### **5.3 Removal of Albumin from Serum With the Bio-Scale Mini Affi-Gel Blue Cartridge**

The Bio-Scale Mini Affi-Gel 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 Section 6.1, Cleaning the Cartridge.

#### **5.4 Purification of Enzymes With the Bio-Scale Mini Affi-Gel Blue Cartridge**

The Bio-Scale Mini Affi-Gel 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 gradient 2 or with a competitive eluant such as a cofactor. Examples of salt gradients include 0.05 to 1.5 M NaCl <sup>3, 4, 5</sup> or 0.0 to 3.0 M KCl.<sup>6, 7, 8</sup> Request technical bulletin 1107 for more information on elution buffers for enzyme purification.
6. Regenerate the cartridge as recommended in Section 6.1, Cleaning the Cartridge.

## Section 6

# Care of the Cartridge

### **6.1 Cleaning the Cartridge**

After each use, both Bio-Scale Mini dye affinity cartridges require thorough cleaning and regeneration to remove bound contaminants. Referring to Table 4

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**

Bio-Scale Mini dye affinity cartridges 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 Cartridge section, then wash with storage buffer.

# 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 US, call Technical Support at 1-800-4BIORAD.

# Section 8

## Ordering Information

### Bio-Scale Mini Cartridges\*

<b>Description</b>	<b>5 x 1 ml</b>	<b>1 x 5 ml</b>	<b>5 x 5 ml</b>
UNOsphere™ Q Support	732-4100	731-4102	731-4104
UNOsphere S Support	732-4110	731-4112	731-4114
Macro-Prep® High Q Support	732-4120	732-4122	732-4124
Macro-Prep High S Support	732-4130	732-4132	732-4134
Macro-Prep DEAE Support	732-4140	732-4142	732-4144
Bio-Gel P-6 Support	—	732-4502	732-4504
Affi-Prep® Protein A Support	732-4600	732-4602	—
Profinity™ IMAC Support	732-4610	732-4612	732-4614
Affi-Gel Blue Support	—	732-4642	732-4644
DEAE Affi-Gel Blue Support	—	732-4632	732-4634

\* Visit [www.bio-rad.com/cartridges/](http://www.bio-rad.com/cartridges/) for current information on prepacked cartridges.

- Larger package sizes of media are available for process-scale chromatography. Inquire with your local Bio-Rad representative.



**Catalog #**  
**Fittings Kits**

**Description**

732-0111	Luer to M6 Adaptor Fittings Kit, includes luer to M6 fitting to connect to an FPLC system
732-0112	Luer to 10-32 Adaptor Fittings Kit, includes luer to polypropylene/PTFE 10-32 fittings to connect 1 cartridge to an HPLC system
732-0113	Luer to BioLogic System Fittings Kit, includes 1/4-28 female to male luer and 1/4-28 female to female luer to connect 1 cartridge to the BioLogic DuoFlow system

# Section 9

## References

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8. Bisson LF and Thorner JJ, Thymidylate synthetase from *Saccharomyces cerevisiae*. Purification and enzymic properties, *J Biol Chem* 256, 12456–12462 (1981)

Cibacron is a trademark of Ciba-Geigy Corporation. FPLC is a trademark of GE Healthcare. Luer-Lok is a trademark of Becton, Dickinson and Co. Triton is a trademark of Union Carbide.

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