Bio-Scale™ Mini Affi-Prep® Protein A Cartridges, 1 and 5 ml

Instruction Manual

Catalog #
732-4600, 732-4602

BIO-RAD

Table of Contents

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-go 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 protein A cartridges are packed with Affi-Prep® Protein A affinity chromatography support. This support is based on hydrophilic spherical polymer beads designed for the purification of monoclonal antibodies. Detailed product information is given in Table 1. See Ordering Information for a listing of the complete Bio-Scale Mini cartridge product line.

Table 1. Protein A Cartridge Specifications

Sizes	1 ml and 5 ml bed volumes
Dimensions	1 ml: 40 mm length x 5.6 mm inner diameter 5 ml: 40 ml length x 12.6 mm inner diameter
Maximum pressure tolerance	45 psi
Recommended flow rates	1 ml: 0.1–0.5 ml/min (25–120 cm/hr) 5 ml: 0.5–2.5 ml/min (24–288 cm/hr)
Fittings	Female luer fitting inlet and male luer fitting outlet
Column material	Polypropylene
Frit material	Polyethylene (HDPE)
Shipping conditions	20% ethanol
Storage recommendations	20% ethanol
Autoclavability	Not autoclavable

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 ID tubing in the pumphead. Adjust the platen pressure screw on the 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. (Use orange lock rings and medium size barb fittings with 1.6 mm tubing.)

*US Patent 5,135,658

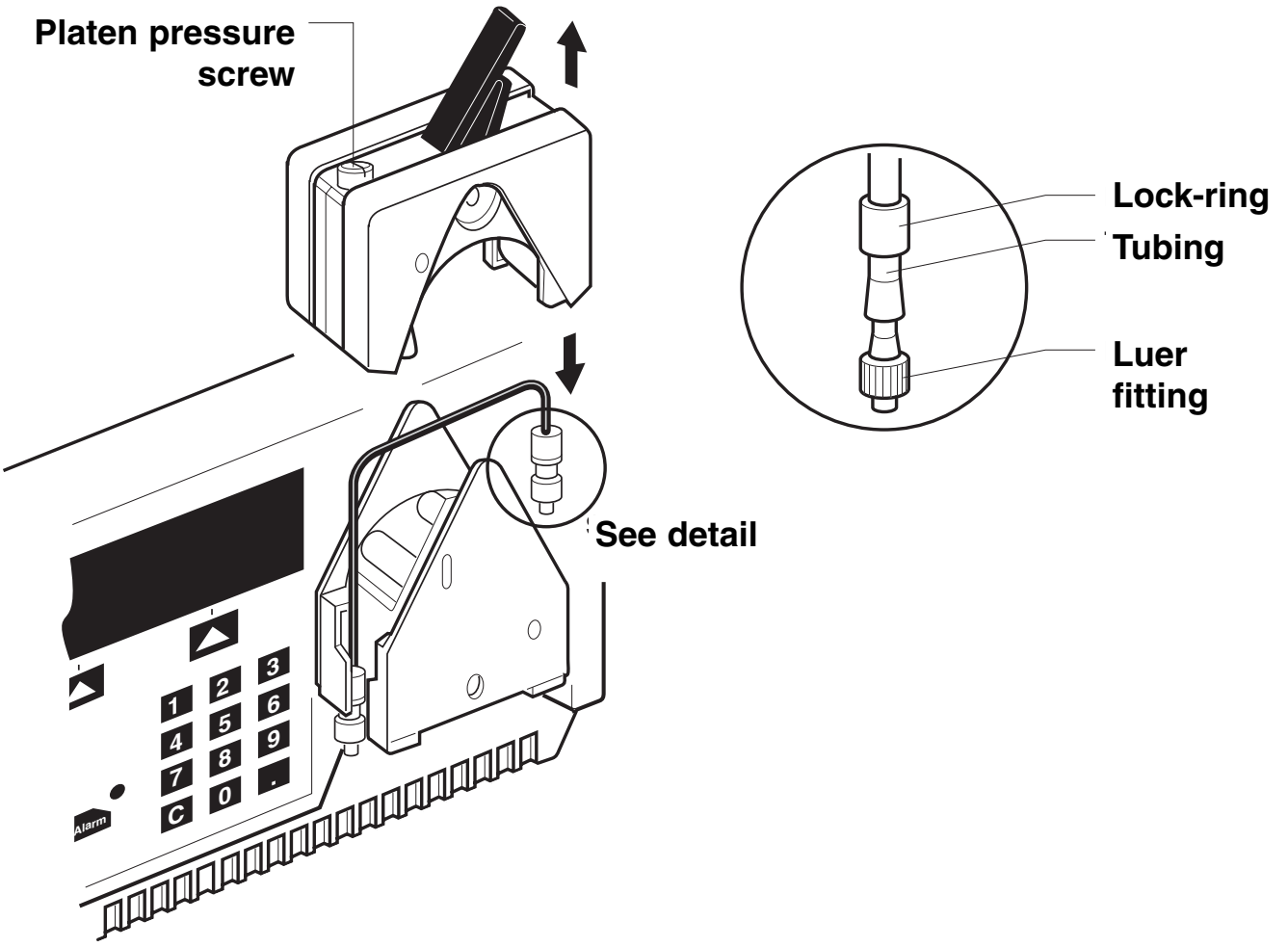

Fig. 1. BioLogic LP system setup.

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).

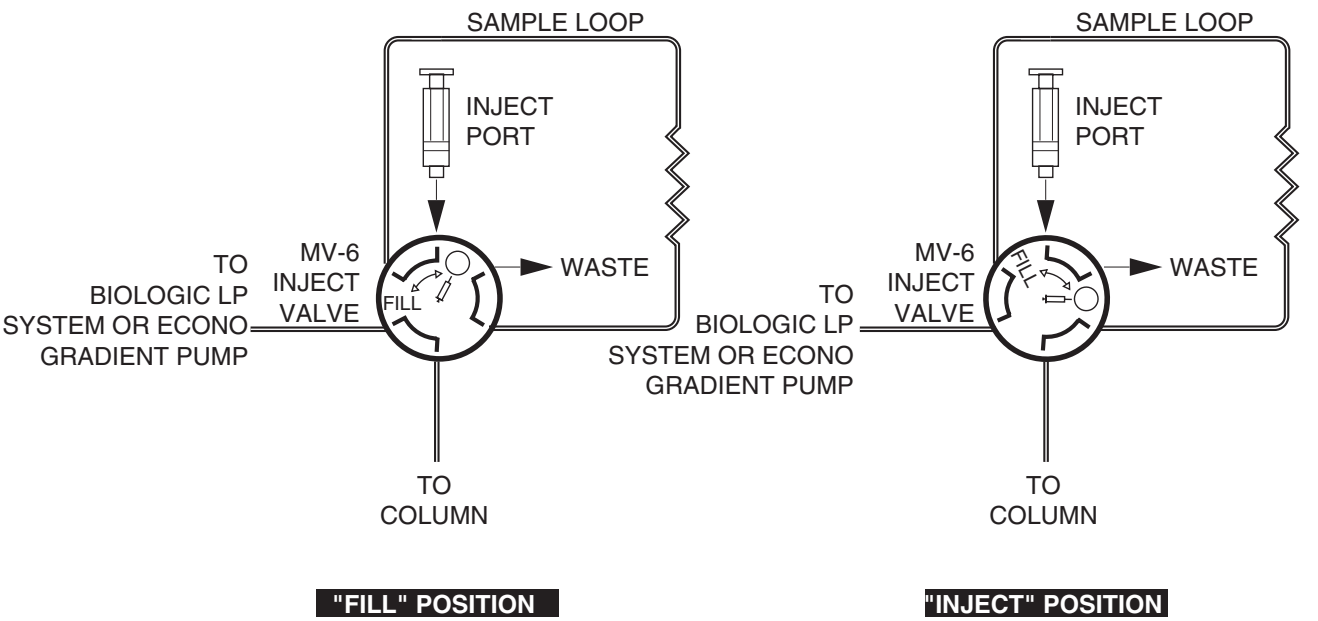

Fig. 2. Connecting to an MV-6 valve.

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 to the Model EM-1 or 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

The 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 fittings 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.

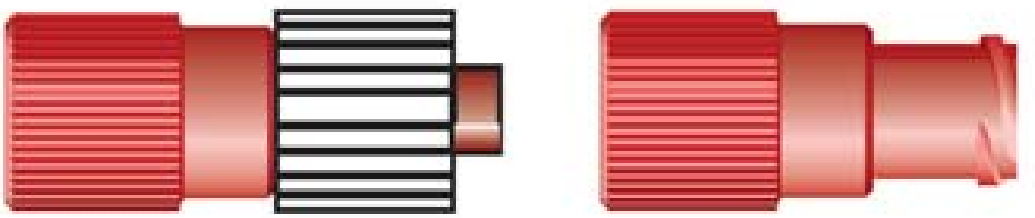

Fig. 4. Luer to 1/4-28 adaptor.

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 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 protein A cartridges contain 20% ethanol 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:

1. Set pump flow rate to 2.0 ml/min.
2. Wash the ethanol from the column with ultrapure water for 5 min.
3. Wash the cartridge with a degassed low-salt buffer (such as 0.5 M sodium phosphate or MAPS II elution buffer) for 5 min.
4. Wash the cartridge with a degassed high-salt buffer (such as 1.5 M sodium phosphate or MAPS II binding buffer) for 10 min.

5. Equilibrate the cartridge for 10 min at 2 ml/min.
6. Reduce the flow rate to the recommended operational flow rate for the specific size cartridge.

4.1 Sample Preparation

Proper adjustment of the pH and ionic strength of the sample is critical for optimal binding. For best results, both the sample pH and ionic strength should be high. This is best accomplished with MAPS II binding buffer, although other buffer systems may be used. (We refer to MAPS II buffers throughout the manual. Protocols for other buffer systems must be determined empirically.) Adjustment of the pH and ionic strength of the sample 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 Bio-Scale Mini P-6 cartridge, Bio-Spin® 6 or Bio-Spin 30 column, Econo-Pac® 10DG desalting column, or Bio-Gel® P-6DG gel filtration gel. The choice of product will depend on the sample volume listed in Table 2.

Table 2. Products for buffer exchange.

Sample Volume	Recommended Product	Use	Catalog #
50–100 µl	Bio-Spin 6 column	Desalting proteins ≥ 6 kD	732-6002
50–100 µl	Bio-Spin 30 column	Desalting proteins ≥ 30 kD	632-6006
100 µl–3 ml	Bio-Scale Mini P-6 cartridge	Desalting proteins ≥ 6 kD	732-4502
Up to 3 ml	Econo-Pac 10DG desalting column	Desalting proteins ≥ 6 kD	732-2010
Unlimited	Bio-Gel P-6DG gel	Desalting proteins ≥ 6kD	150-0738

Ascites fluid should be diluted 1:2 with MAPS II binding buffer. Higher concentrations of binding buffer can enhance the binding of low-affinity antibodies.

Tissue culture supernatants may be concentrated to approximately 5 mg of immunoglobulin per ml, and then diluted 1:2 with MAPS II binding buffer. For large volume samples where further dilution is not desired, we recommend adding dry MAPS II binding buffer salts directly to the sample (31.4 g buffer salts/100 ml sample) instead of diluting the sample with prepared buffer. All samples should be filtered through a 0.45 µm filter.

4.2 MAPS II Buffers

MAPS II buffers provide a dramatic improvement in protein A affinity methods for the purification of mouse IgG1 antibodies from ascites fluid. Capacity is significantly increased to 8–10 mg IgG1 per ml of support, which is 8–10 times higher than that obtained with published methods. (Ey, et al.1978, Bigbee, et al, 1983).

All subclasses of mouse IgG in addition to other species (human, rabbit, bovine, goat) of IgG can be purified with the MAPS buffers.

4.3 General Purification Protocol

Equilibrate the Bio-Scale Mini Protein A cartridge with 7–13 column volumes (CVs) of MAPS II binding buffer and adjust the flow rate to a flow rate suitable for antibody binding (0.1–0.5 ml/min recommended for the 1 ml cartridge and 0.5–1.0 ml/min for the 5 ml cartridge). Binding efficiency of immunoglobulin to the Bio-Scale Mini Protein A cartridge is optimal at the lower flow rates.

After adjusting the flow rate, apply the prepared sample to the cartridge. Wash the cartridge with 10–20 CVs of binding buffer to remove all of the unbound contaminating components.

Elute the immunoglobulin with 5-10 column volumes of MAPS II elution buffer. Elute with an additional 10 column volumes of elution buffer to insure total removal of immunoglobulin. Neutralize the eluted sample immediately after elution with 1 M Tris-HCl, pH 8.8, or 1 N NaOH. Prolonged exposure of the purified immunoglobulin to acid pH should be avoided. Regenerate the Bio-Scale Mini Protein A cartridge with 7 column volumes of 50% methanol after each use and equilibrate with 7 to 13 column volumes of binding buffer if the cartridge is to be used. The pH of the cartridge effluent should be 9.0 when equilibrated.

4.4 Scaling Up the Separation

For quick scale-up, two or three cartridges can be connected in series. The Affi-Prep Protein A support is available in larger amounts, from 25 ml to bulk quantities, for scaling up methods developed using the cartridges. In addition, Bio-Rad carries an extensive line of empty chromatography columns.

Section 5
Care of the Cartridge

5.1 Cleaning

After each use, a Bio-Scale Mini Protein A 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 7 column volumes of 50% methanol at 3 ml/min.
2. Wash the cartridge with 5–8 column volumes of 0.1 N NaOH.

3. Equilibrate the cartridge with at least 7 column volumes of binding buffer.

The 0.1 N NaOH wash will remove any bound phenol red from the cartridge. For complete sanitation (i.e., removal of endotoxins and DNA), the cartridge can be washed with 1.0 N NaOH.

5.2 Storage

Bio-Scale Mini Protein A cartridges should be stored in 20% ethanol. Wash cartridges with ultrapure water, then purge with storage buffer.

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

Section 7
Ordering Information

Description	5 x 1 ml	1 x 5 ml	5 x 5 ml
Bio-Scale Mini Cartridges*			
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® DEAE Blue Support	—	732-4632	732-4634
Affi-Gel Blue Support	—	742-4642	732-4644

* Visit 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.

Fittings Kits

Catalog #	Description
732-0111	Luer to M6 Adaptor Fittings Kit, includes luer to M6 fitting to connect to FPLC system
732-0112	Luer to 10-32 Adaptor Fittings Kit, includes luer to polypropylene/PTFE 10-32 fittings to connect 1 cartridge to 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
153-6161	Protein A MAPS II Binding Buffer, makes 5 L

Section 8 References

Bigbee WL et al., Monoclonal antibodies specific for the M- and N- forms of human glycophorin A, Mol Immunol 20, 1353–62 (1983)

Bio-Rad Laboratories, Inc.: Antibody purification using the Econo-Pac protein A cartridge and the Econo system, Bio-Rad bulletin 1836 (1995)

Ey Pl et al., Isolation of pure IgGl, IgG2a and IgG2b immunoglobulins from mouse serum using Protein A-sepharose, Immunochem 15, 429–36 (1978)

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