
Bio-Scale™ Mini
UNOsphere SUPrA™
Cartridges, 1 and 5 ml

Instruction Manual

Catalog #

732-4200

732-4201

732-4202

BIO-RAD

For technical support, contact your local Bio-Rad office, or in the U.S., call 1-800-4BIORAD (1-800-424-6723).

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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 most commonly used for protein purification. The polypropylene Luer fittings and internal sealing surfaces assure leak-free operation at pressures up to 45 psi. The cartridges are convenient, disposable, and supplied ready for use. Cartridges are available for a variety of chromatographic techniques including desalting, ion exchange, and affinity chromatography. See Ordering Information (Section 7) for a listing of the complete Bio-Scale Mini cartridge product line.

UNOsphere SUPrA™ medium is a chromatographic support based on recombinant protein A. The media are designed for process-scale purification of monoclonal antibodies. The protein A ligand is produced in *E. coli* without the use of material from animal origin. The UNOsphere base bead is a macroporous polymeric bead that is designed for robust and scaleable applications. See Tables 1 and 2 on pages 4–5 for the technical description of the product.

UNOsphere SUPrA media are built on the proven UNOsphere base bead, which insures an easy scale-up path for process applications. The outstanding flow pressure performance of UNOsphere chromatography media allow its use in process applications without concern for exceeding the pressure limits of the media or chromatography system. The flow characteristics of UNOsphere SUPrA packed in a large column format are shown in Figure 1.

UNOsphere SUPrA affinity chromatography media come with full regulatory support and are backed by the support of Bio-Rad's global application and development team.

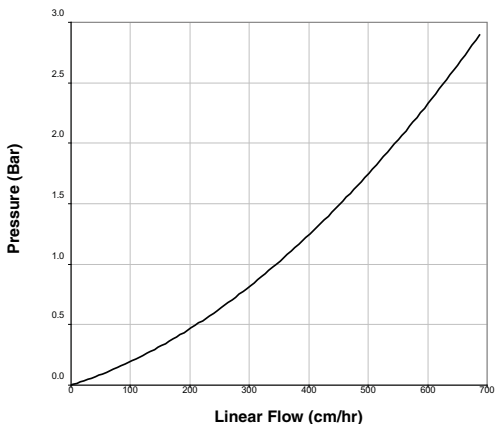


Fig. 1. Flow performance of UNOsphere SUPrA media in Bio-Rad's EasyPack™ column (20 x 20 cm) packed to 13.1% axial compression.

Table 1. Bio-Scale Mini UNOsphere SUPRA Cartridge Specifications.

Sizes	1 and 5 ml bed volumes
Dimensions	1 ml: 40 x 5.6 mm, length x inner diameter 5 ml: 40 x 12.6 mm, length x inner inner diameter
Maximum pressure tolerance	45 psi
Recommended flow rates	1 ml: 1–2 ml/min (240–480 cm/hr) 5 ml: 5–10 ml/min (140–480 cm/hr)
Maximum flow rate	1 ml: 3 ml/min (730 cm/hr) 5 ml: 15 ml/min (722 cm/hr)
Fittings	Female Luer fitting inlet and male Luer fitting outlet
Column material	Polypropylene
Frit material	Polyethylene (HDPE)
Shipping condition	20% ethanol
Storage recommendation	20% ethanol
Autoclavability	Not autoclavable

Table 2. Technical Description of UNOsphere SUPrA Affinity Chromatography Media.

Composition	Highly crosslinked polyacrylamide polymer	
Particle size range	53–61 μm	
Ligand	Recombinant protein A	
Coupling chemistry	Epoxy	
Dynamic binding capacity*	150 cm/hr	30 ± 3 mg/ml
	300 cm/hr	25 ± 2 mg/ml
	450 cm/hr	20 ± 2 mg/ml
Chemical stability**	10 mM hydrochloric acid	
	6 M guanidine hydrochloride	
	0.1 M arginine (pH 2.8)	
	0.1 M citrate (pH 2.8)	
	0.1 M glycine (pH 2.8)	
Working pH range	3–11	
Cleaning-in-place (CIP)	6 M guanidine hydrochloride	
	10 mM hydrochloric acid	
	0.1 M sodium hydroxide	
	1 M acetic acid/20% ethanol	
Recommended mobile phase velocity range	100–600 cm/hr	
Temperature stability	4–40°C	
Delivery conditions	50% slurry in 20% ethanol	
Storage conditions	4–8°C	

* Minimum 20 mg/ml at 300 cm/hr; 10% breakthrough capacity determined with 1.0 mg/ml polyclonal human IgG in 1.1 x 10 cm column.

** No significant change in chromatographic performance after 24-hour storage at room temperature.

Section 2

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

The Bio-Scale™ Mini cartridges are ideal for use with Bio-Rad's BioLogic™ LP system, Econo gradient pump, and 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 lock fittings on the cartridge.

1. Install 1.6 mm ID tubing in the pumphead. Adjust 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.

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

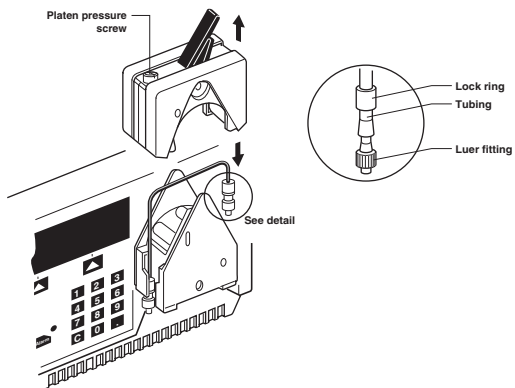


Fig. 1. Biologic LP setup.

2. To maximize gradient accuracy and 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.)

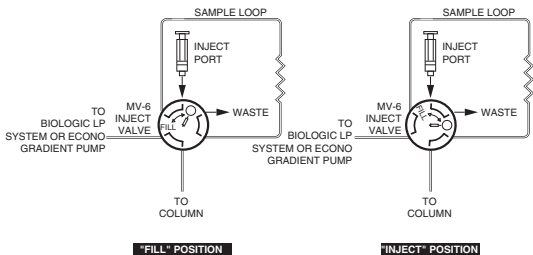


Fig. 2. Connecting to a MV-6 valve.

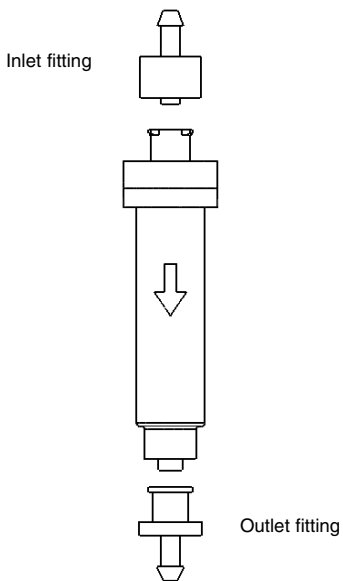


Fig. 3. Column and fittings.

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.
4. Connect the cartridge outlet to the 1.6 mm ID tubing leading to the BioLogic LP optics module 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 fitting to the 1.6 mm ID tubing, then connect to the bottom of the male Luer on the Bio-Scale Mini cartridge.

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 Bio-Scale Mini cartridge to BioLogic system fittings kit (catalog #732-0113) includes ¼–28 female to male Luer and ¼–28 female to female Luer to connect one Bio-scale Mini cartridge to the BioLogic DuoFlow system.



3.2 HPLC Systems

The Luer to 10–32 adaptor fittings kit (catalog #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 in 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 in 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.

Section 4

Getting Started

4.1 Screening Buffers and Conditions for UNOsphere SUPrA™

Buffers

A1: 0.02 M sodium phosphate, 0.02 M sodium citrate, pH 7.5.

A2: 0.02 M boric acid, 0.02 M sodium phosphate, 0.02 M sodium citrate, 1.0 M sodium sulfate, pH 9.0.

B: 0.02 M sodium citrate, 0.1 M sodium chloride, pH 2.5.

Conditions

Equilibrate column: with 10 CV buffer A1 or A2 (see note below).

Inject: MAb sample either as is or diluted 1:10 into buffer A1 or A2 (see below).

Wash: buffer A1 or A2 until effluent absorbance returns to baseline.

Elute: in a 10 CV linear gradient to 100% B, or Desired % buffer B.

Strip: with 5 CV buffer B.

Use buffer A1 for binding human and guinea pig IgG. Use buffer A2 for all others. The recommended column equilibration interval is excessive under most conditions, but should be used as a default until specific equilibration requirements are established for your particular system. In addition, be aware of solubility limitations of antibodies that require high salt concentrations (A2 buffer) for binding.

Characterize product solubility thoroughly under loading conditions. If the antibody fails to remain fully soluble for the longest possible duration from equilibration to completion of sample load when adjusted to load conditions, then load by using online dilution technique.

Initial selectivity screening should be conducted with a linear gradient. Knowledge of a monoclonal's subclass may suggest a particular range of conditions, but variation from one monoclonal to another is sufficient to risk incomplete or no elution.

The choice of citrate for the low pH buffer is predicated on the broad pH range achievable with phosphate/citrate systems. If a higher pH range is required, add boric acid to the binding buffer.

(Note: if CHT™ ceramic hydroxyapatite is used as the subsequent polishing column, once scouting is finished replace citrate with glycine or other nonchelating salt for further process development. Citrate buffers are incompatible with CHT.)

4.2 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 ≤ 45 psi.

Bio-Scale™ Mini cartridges are available in 1 and 5 ml cartridge format. The UNOsphere SUPrA media are also available in larger amounts from 25 ml bottles to bulk quantities.

Section 5

Care of the Cartridge

5.1 Cleaning

During operation it is recommended that the column bed is periodically cleaned to remove bound substances that can adversely impact the separation performance of the column. The accumulated substances fall into two general categories: a) difficult to remove precipitated or denatured substances, and b) substances that are hydrophobically bound to the column bed. To ensure that all bound substances are released and washed out of the column, the following cleaning-in-place (CIP) protocols are recommended.

CIP Protocols

The following protocols are suggested to remove precipitated or denatured substances from the bed.

Wash the bed with 2–5 column volumes in reverse flow with one of the following solutions:

- 6 M guanidine hydrochloride
- 10 mM hydrochloric acid
- 0.1 M sodium hydroxide
- 1 M acetic acid/20% ethanol

Followed by a reverse flow wash with at least 5 column volumes of binding buffer, neutral pH (7–8).

To remove any hydrophobically bound substances from the bed, wash the column with 2–5 column volumes in reverse flow of a nonionic surfactant/detergent, followed by a reverse-flow wash with at least 5 column volumes of neutral pH binding buffer.

Suggested contact time per cycle is 15 min at room temperature.

5.2 Sanitization

To reduce the potential for microbial contamination of the cartridge, the column can be periodically washed with a solution consisting of 0.1 M sodium hydroxide. Allow to stand for 1 hour, then wash with buffer until a neutral pH is reached.

5.3 Storage

To store UNOsphere SUPrA™ for long periods, equilibrate the media with a 20% ethanol/water solution and store at 4°C.

5.4 Autoclaving

Bio-Scale™ Mini cartridges are not autoclavable.

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., call technical support at 1-800-4BIORAD.

Section 7

Ordering Information

Bio-Scale™ Mini Cartridges*

Description	5 x 1 ml	1 x 5 ml	5 x 5 ml	1 x 1 ml
UNOsphere™ Q Support	732-4100	731-4102	731-4104	
UNOsphere S Support	732-4110	731-4112	731-4114	
UNOsphere Rapid S	732-4400	732-4401	732-4402	
UNOsphere SUPrA	732-4201	732-4202	—	732-4200
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	—	732-4642	732-4644	

* For the most up-to-date list of cartridge offerings, please visit us online at www.bio-rad.com/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 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 ¼–28 female to male Luer and ¼–28 female to female Luer to connect one cartridge to the BioLogic DuoFlow™ system

Section 8

References

1. Harris ELV and Angal S, Protein Purification Methods: A Practical Approach, IRL Press, Oxford (1989)
2. Scopes RK, Protein Purification: Principles and Practice (Second Edition), Springer-Verlag, New York (1987)
3. Snyder LR and Kirkland JJ, Introduction to Modern Liquid Chromatography (Second Edition), Wiley, New York (1979)
4. Gagnon P, Avoiding Instrument-associated Aberrations in Purification Scale-up and Scale-down, BioPharm 10, 42–45 (1997)
5. Gagnon P, Purification Tools for Monoclonal Antibodies Validated Bio Systems (1996)

FPLC is a trademark of GE Healthcare Group Companies. Luer-Lok is trademark of Becton, Dickinson and Co. Triton is a trademark of Union Carbide Corp. Upchurch is a trademark of Upchurch Scientific.

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