
Bio-Scale™ Mini
UNOsphere™ Q, S and Rapid S
Cartridges, 1 and 5 ml

Instruction Manual

Catalog #

732-4100, 732-4102, 732-4400,
732-4104, 732-4110, 732-4401,
732-4112, 732-4114, 732-4402

BIO-RAD

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

Table of Contents

Section 1	Introduction.....	1
Section 2	Connection to Bio-Rad's Low-Pressure Chromatography Instruments	5
Section 3	Connection to Other Liquid Chromatography Systems	10
	3.1 BioLogic DuoFlow™ Systems.....	11
	3.2 HPLC Systems	11
	3.3 FPLC Systems	12
Section 4	Preparing a Cartridge For Use	13
	4.1 Sample Preparation	14
	4.2 General Purification Protocol ..	15
	4.3 Scaling Up the Separation	16
Section 5	Care of the Cartridge.....	19
	5.1 Autoclaving	20
	5.2 Storage	20

Section 6	Technical Assistance	21
Section 7	Ordering Information.....	22
Section 8	References	24

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. The design of the 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

The Bio-Scale Mini UNOsphere™ Q, S, and Rapid S cartridges are packed with UNOsphere ion exchange supports. These supports are based on hydrophilic spherical, polymeric beads designed for the purification of proteins, nucleic acids, viruses, plasmids, and other macromolecules. UNOsphere beads are designed to provide high capacity, low back-pressure, and high productivity. Detailed product information is given in Tables 1 and 2.

Table 1 Bio-Scale Mini UNOsphere Q, S, & Rapid S Cartridge Specifications.

Sizes	1 and 5 ml bed volumes
Dimensions	1 ml: 40 x 5.6 mm inner diameter 5 ml: 40 x 12.6 mm 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: 4 ml/min (970 cm/hr) 5 ml: 20 ml/min (963 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 UNOsphere Q, S, and rapid S Specifications

	UNOsphere Q	UNOsphere S	UNOsphere Rapid S
Type of ion exchanger	Strong anion	Strong cation	Strong cation
Functional group	-N ⁺ (CH ₃) ₃	-SO ₃	-SO ₃
Total ionic capacity	120 µeq/ml Ni ²⁺	260 µeq/ml	140 µeq/ml
Dynamic binding capacity*			
At 150 cm/hr	180 mg BSA/ml	60 mg IgG/ml	60 mg/ml
At 600 cm/hr	125 mg BSA/ml	30 mg IgG/ml	30 mg/ml
Shipping counterion	Cl ⁻	Na ⁺	Na ⁺
Median particle size	120 µm	80 µm	100 µm
Recommended linear flow rate	50–1,200 cm/hr	50–1,200 cm/hr	50–800 cm/hr
Chemical stability			
1.0 M NaOH (20°C)	Up to 10,000 hr	Up to 10,000 hr	Up to 800 hr
1.0 M HCl (20°C)	Up to 200 hr	Up to 200 hr	Up to 200 hr
pH stability	1–14	1–14	1–14
Antimicrobial agent	20% ethanol	20% ethanol	20% ethanol
Regeneration	1–2 M NaCl	1–2 M NaCl	1–2 M NaCl
Storage conditions	20% ethanol or 0.1 M NaOH	20% ethanol or 0.1 M NaOH	20% ethanol or 0.1 M NaOH

* 10% breakthrough capacity determined with 4.5 mg/ml human IgG (UNOsphere Rapid S) and 2.0 mg/ml BSA (UNOsphere Q) in a 1.1 x 20 cm column.

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.)

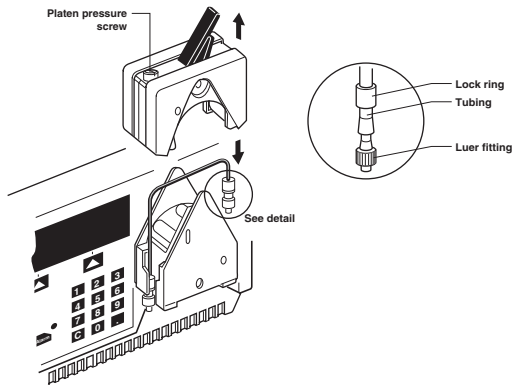


Fig. 1. Biologic LP setup

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.)

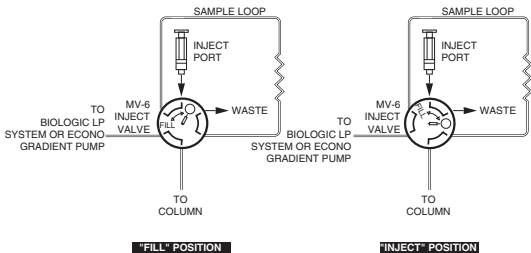


Fig. 2. Connecting to a MV-6 valve.

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 to the 1.6 mm ID tubing, then connect to the bottom of the male Luer on the Bio-Scale mini cartridge.

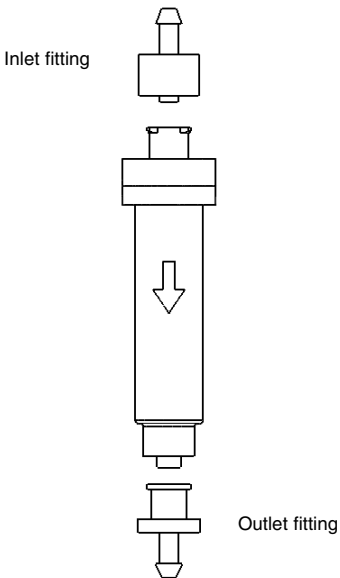


Fig. 3. Column and fittings.

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 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.

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 Luer fitting to the 1/4–28 metric adaptors. Attach the adaptor with the male Luer to the column inlet line of the FPLC system and the adaptor with the female luer to the FPLC column outlet. To prevent tubing or cartridge failure, do not exceed the maximum recommended flow rate.

Section 4

Preparing a Cartridge For Use

Bio-Scale™ Mini UNOsphere™ cartridges contain 20% ethanol v/v as the storage solution. The fully hydrated support is ready to use after equilibrating the cartridge in the buffer of choice. To perform a 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 3.0 ml/min (731 cm/hr) for the 1 ml cartridge or 6.0 ml/min (288 cm/hr) for the 5 ml cartridge.
2. Wash the cartridge with degassed low-salt buffer for 2 min.
3. Wash the cartridge with degassed high-salt buffer for 5 min.

4. Equilibrate the cartridge with low-salt buffer for 5 min.
5. Reduce the flow rate to the rate that will be used in the purification protocol.

4.1 Sample Preparation

Proper pH and ionic strength is 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 the Bio-Scale Mini P6 cartridge, Bio-Spin® 6 or Bio-Spin 30 columns, Econo-Pac® 10DG desalting columns, or Bio-Gel® P-6DG gel filtration gel. The choice of product will depend on sample volume. All samples should be filtered through a 0.45 µm filter prior to cartridge application.

Table 1 Products for Buffer Exchange.

Sample Volume	Recommended Product	Use	Catalog #
50–100 µl	Bio-Spin 6 column	Desalting proteins 6 kD	732-6000
50–100 µl	Bio-Spin 30 column	Desalting proteins 30 kD	732-6004
100 µl–3 ml	Bio-Scale Mini P6 cartridge	Desalting proteins 6 kD	732-4502
Up to 3 ml	Econo-Pac 10DG desalting columns	Desalting proteins 6 kD	732-2010
Unlimited	Bio-Gel P-6DG gel	Desalting proteins 6 kD	150-0738

4.2 General Purification Protocol

Ion exchange chromatography is usually performed using increasing salt gradients or pH gradients to elute the sample components. For best results, and increased cartridge life, samples and buffers should be degassed and filtered through a 0.45 µm filter.

Common buffers for cation exchange chromatography are listed in Table 3.

An appropriate starting point for purifying samples is a linear gradient from 0–0.4 M NaCl spanning 1–20 column volumes at 120 cm/hr, 0.5 ml/min for the 1 ml cartridge, and 2.5 ml/min for the 5 ml cartridge. The separation can be optimized by changing the gradient profile. At the end of each run the cartridge can be regenerated with 1.0 M NaCl followed by starting buffer. Return to the desired flow rate and proceed with the next separation.

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 ≤ 45 psi. Bio-Scale Mini cartridges are available in 1 and 5 ml cartridge format. The UNOsphere Q, S, and Rapid S ion exchange media are also available in larger

amounts, from 25 ml bottles to bulk quantities for scaling-up methods developed using the cartridges. UNOsphere Q, S, and Rapid S media are fully supported with regulatory support files. In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory to process scale.

Table 3 Common Buffers for Ion Exchange Chromatography. ^{1,2,3}

Type of Buffering

Cation	Ion Exchanger Buffer Range
Acetic acid	4.8–5.2
Citric acid	4.2–5.2
HEPES	7.6–8.2
Lactic acid	3.6–4.3
MES	5.5–6.7
MOPS	6.5–7.9
Phosphate	6.7–7.6
PIPES	6.1–7.5
Pivalic acid	4.7–5.4
TES	7.2–7.8
Tricine	7.8–8.9
Anion	
Bicine	7.6–9.0
Bis-Tris	5.8–7.2
Diethanolamine	8.4–8.8
Diethylamine	9.5–11.5
L-Histidine	5.5–6.0
Imidazole	6.6–7.1
Pyridine	4.9–5.6
Tricine	7.8–8.9
Triethanolamine	7.3–8.0
Tris	7.5–8.0

Section 5

Cleaning-in-Place (CIP) and Sanitation

If a column no longer yields reproducible results, the media may require thorough CIP and sanitation to remove strongly bound contaminants. Acceptable CIP agents include 25% acetic acid, 8 M urea, 1% Triton X-100, 6 M potassium thiocyanate, 70% ethanol, 30% isopropyl alcohol, 1 N HCl, 1 N NaOH, and 6 M guanidine hydrochloride.

1. Sanitize the support in the column with 2–4 bed volumes of 1.0 M NaOH at 50–100 cm/hr while maintaining a minimum contact time of 40 min.
2. To reequilibrate the column, wash the column with 2–4 bed volumes of 0.5–2 M NaCl solution (containing 50–100 mM buffer).

3. If lipid removal is required, the column may be washed with a 20–50% ethanol solution at 50 cm/hr.

5.1 Autoclaving

Bio-Scale Mini[®] cartridges are not autoclavable.

5.2 Storage

After washing the cartridges with deionized water, Bio-Scale Mini ion exchange cartridges should be purged and stored with PBS, containing 0.05% NaN₃, or in 20% v/v ethanol solution, and capped for extended storage.

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 USA, 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
UNOsphere™ Q Support	732-4100	731-4102	731-4104
UNOsphere S Support	732-4110	731-4112	731-4114
UNOsphere Rapid S Support	732-4400	732-4401	732-4402
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)

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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