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Bio-Scale Mini®  
UNOsphere Q & S  
Cartridges, 1 and 5 ml

Instruction Manual

Catalog #

732-4100, 732-4102

732-4104, 732-4110

732-4112, 732-4114

**BIO-RAD**



# Table of Contents

|           |  |    |
|-----------|--|----|
| Section 1 | Introduction .....   | 1  |
| Section 2 | Connection to Bio-Rad's Low-<br>Pressure Chromatography<br>Instruments ..... | 5  |
| Section 3 | Connection to Other Liquid<br>Chromatography Systems .....                   | 10 |
| 3.1       | BioLogic DuoFlow™<br>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       | Cleaning.....  | 19 |

|           |     |                            |    |
|-----------|-----|----------------------------|----|
|           | 5.2 | Autoclaving .....          | 21 |
|           | 5.3 | Storage .....              | 21 |
| Section 6 |     | Technical Assistance ..... | 22 |
| Section 7 |     | Ordering Information.....  | 23 |
| Section 8 |     | References .....           | 25 |

# 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. They are easy-to-use, prepacked chromatographic cartridges for fast, reproducible chromatographic separations. Cartridges are available for a variety of chromatographic techniques including desalting, ion exchange, and affinity chromatography. See Ordering Information 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 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 Cartridge Specifications**

|                            |   |
|----------------------------|---|
| Sizes                      | 1 ml and 5 ml bed volumes   |
| Dimensions                 | 1 ml: 40 mm length x 5.6 mm inner diameter<br>5 ml: 40 mm length 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)<br>5 ml: 5–10 ml/min (140–480 cm/hr)                           |
| Maximum flow rate          | 1 ml: 6 ml/min (1440 cm/hr)<br>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 Specifications

|                                |   |                              |
|--------------------------------|---|------------------------------|
| Type of ion exchanger          | Strong anion                                    | Strong cation                |
| Functional group               | -N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub> | -SO <sub>3</sub>             |
| Total ionic capacity           | 120 µeq/ml Ni <sup>2+</sup>                     | 260 µeq/ml                   |
| Dynamic binding capacity*      |   |                              |
| At 150 cm/hr                   | 180 mg BSA/ml                                   | 60 mg IgG/ml                 |
| At 600 cm/hr                   | 125 mg BSA/ml                                   | 30 mg IgG/ml                 |
| Shipping counterion            | Cl <sup>-</sup>                                 | Na <sup>+</sup>              |
| Median particle size           | 120 µm  | 80 µm                        |
| Recommended linear flow rate** | 50–1,200 cm/hr                                  | 50–1,200 cm/hr               |
| Chemical stability             |   |                              |
| 1.0 M NaOH (20°C)              | Up to 10,000 hr                                 | Up to 10,000 hr              |
| 1.0 M HCl (20°C)               | Up to 200 hr                                    | Up to 200 hr                 |
| Volume changes                 |   |                              |
| pH 4–10                        | ≤5%   | ≤5%                          |
| 0.01–1.0 M NaCl                | ≤5%   | ≤5%                          |
| pH stability                   | 1–14  | 1–14                         |
| Antimicrobial agent            | 20% ethanol                                     | 20% ethanol                  |
| Regeneration                   | 1–2 M NaCl                                      | 1–2 M NaCl                   |
| Storage conditions             | 20% ethanol or<br>0.1 M NaOH                    | 20% ethanol or<br>0.1 M NaOH |

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

\*\* UNOsphere packed into a 20 cm bed height and run at 1,200 cm/hr generates <2 bar backpressure.

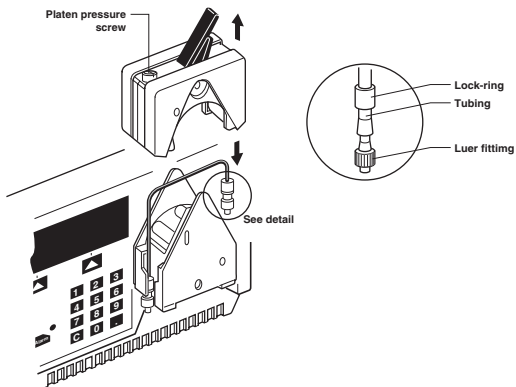
# Section 2

## 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.

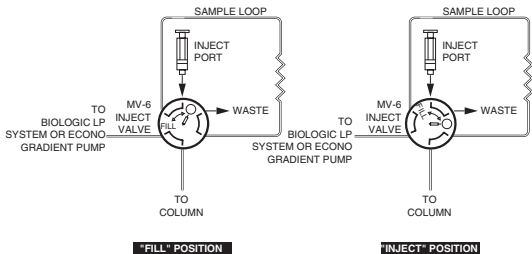
1. Install 1.6 mm ID tubing in the pumphead. Adjust platen pressure screw (on pumphead) — Using a screwdriver or coin, turn the screw counterclockwise as far as it will go, then turn clockwise three full turns. Assemble with fittings and lock rings as shown in Figure 1.

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



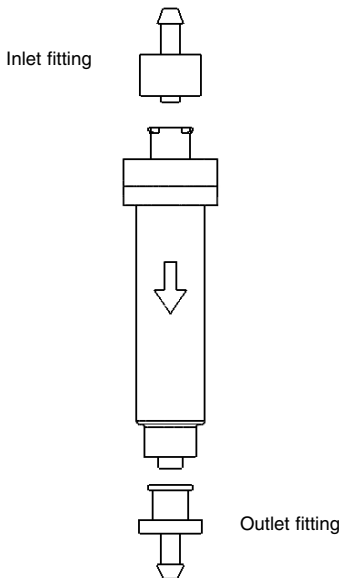
**Figure 1. Biologic LP setup**

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).



**Figure 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.



**Figure 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 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.



### 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 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 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 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 luers 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 one 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 Q and UNOsphere S 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 that which 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'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 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 2. Products for Buffer Exchange**

| <b>Sample Volume</b> | <b>Recommended Product</b>       | <b>Use</b>                      | <b>Catalog #</b> |
|----------------------|----------------------------------|---------------------------------|------------------|
| 50–100 $\mu$ l       | Bio-Spin 6 column                | Desalting proteins $\geq 6$ kD  | 732-6000         |
| 50–100 $\mu$ l       | Bio-Spin 30 column               | Desalting proteins $\geq 30$ kD | 732-6004         |
| 100 $\mu$ l–3 ml     | Bio-Scale Mini P6 cartridge      | Desalting proteins $\geq 6$ kD  | 732-4502         |
| Up to 3 ml           | Econo-Pac 10DG desalting columns | 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**

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  $\mu$ m filter.

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

An appropriate starting point for purifying samples is a linear gradient from 0 to 0.4 M NaCl spanning 1 to 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  $\leq 45$  psi. Bio-Scale Mini cartridges are available in 1 ml and a 5 ml cartridge format. The UNOsphere Q and 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 media are fully supported with Regulatory Support Files. In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory scale to process scale.

### **Table 3. Common Buffers for Ion Exchange Chromatography** <sup>1,2,3</sup>

#### **Type of Buffering**

| <b>Cation</b> | <b>Ion Exchanger Buffer Range</b> |
|---------------|-----------------------------------|
|---------------|-----------------------------------|

|              |         |
|--------------|---------|
| 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

## Care of the Cartridge

### **5.1 Cleaning**

After repeated use, an ion exchange cartridge may require thorough cleaning and regeneration to remove bound contaminants. Most bound contaminants may be removed by following the procedure below:

- Step 1. Wash the cartridge with 5 column volumes (1 ml/min for the 1 ml or 5 ml/min for the 5 ml) of 1 M NaOH.
- Step 2. Wash with 3 column volumes (1 ml/min for the 1 ml or 5 ml/min for the 5 ml) of deionized water or starting buffer.
- Step 3. Wash with 3 column volumes (1 ml/min for the 1 ml or 5 ml/min for the 5 ml) of high salt buffer.
- Step 4. Equilibrate the cartridge with at least

5 column volumes (1 ml/min for the 1 ml or 5 ml/min for the 5 ml) of starting buffer.

If bound contaminants persist after following the procedure above, use one of the alternative procedures below:

### **Wash Alternative 1**

Perform wash alternative 1 in place of step 1, the NaOH wash. Continue with steps 2–4 listed on page 19.

### **Wash Alternative 2**

Perform step 2 above. Wash with any of the following alternative cleaning buffers listed below. Perform a wash alternative 2 with any one of the following alternative buffers in place of step 1, the NaOH wash. Continue with steps 2–4 listed on page 19.

- 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
- 6 M guanidine hydrochloride

## **5.2 Autoclaving**

Bio-Scale Mini cartridges are not autoclavable.

## **5.3 Storage**

After washing the cartridges with deionized water, Bio-Scale Mini ion exchange cartridges should be purged and stored with PBS, containing 0.05%  $\text{NaN}_3$ , 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\*

| <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® 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/](http://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**

| <b>Catalog #</b> | <b>Description</b>   |
|------------------|--|
| 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/Teflon 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 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. Luer-Lok is trademark of Becton, Dickinson and Co. Triton is a trademark of Union Carbide.





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