
Bio-Scale™ Nuvia™ S Cartridges

1 and 5 ml

Instruction Manual

Catalog #732-4420

732-4421

732-4422

732-4423

BIO-RAD

Bio-Rad Technical Support

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Triton is a trademark of Union Carbide Corporation. Upchurch Scientific is a trademark of IDEX Health and Scientific LLC.

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Section 1

Introduction

Bio-Scale™ Mini cartridges have a patented* double-wall design that provides extra durability and allows easy, reliable runs with the aqueous buffers most commonly used for protein purification. The polypropylene luer fittings and internal sealing surfaces ensure 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 Section 6, Ordering Information, for the complete Bio-Scale Mini cartridge product line.

*U.S. patent 7,208,087.

The design of the Bio-Scale Mini cartridges offers:

- Convenience — the cartridge is ready for equilibration in the buffer of choice
- Versatility — luer fittings allow connection to any chromatography system or directly to a Luer-Lok syringe

Bio-Scale Mini Nuvia™ S cartridges are packed with Nuvia S ion exchange support. This ultra-high capacity ion exchange support is built on the UNOsphere™ platform and is designed for purification of proteins, nucleic acids, viruses, plasmids, and other macromolecules. Nuvia S support may be used at any stage of the purification process.

Table 1. Bio-Scale Mini Nuvia S cartridge specifications.

Sizes	1 and 5 ml bed volumes
Dimensions	1 ml: 40 x 5.6 mm inner diameter (ID) 5 ml: 40 x 12.6 mm ID
Max pressure tolerance	45 psi
Recommended flow rates	1 ml: 1–2 ml/min (240–280 cm/hr) 5 ml: 5–10 ml/min (140–480 cm/hr)
Max flow rate	1 ml: 4 ml/min (970 cm/hr) 5 ml: 20 ml/min (963 cm/hr)
Fittings	Female luer fitting inlet Male luer fitting outlet
Column material	Polypropylene
Frit material	Polyethylene (HDPE)
Shipping conditions	20% ethanol + 0.1 M NaCl
Storage	20% ethanol or 0.1 N NaOH
Autoclavability	Not autoclavable

Table 2. Nuvia S media specifications.

Type of exchanger	Strong cation
Functional group	$-\text{SO}_3^-$
Total ionic capacity	90–150 $\mu\text{eq/ml}$
Dynamic binding capacity [*]	≥ 110 mg/ml (300 cm/hr)
Shipping counterion	Na^+
Median particle size	85 ± 15 μm
Recommended linear flow rate ^{**}	50–600 cm/hr
Chemical stability	
1.0 N NaOH (20°C)	Up to 1 week
0.1 N NaOH (20°C) ^{***}	Up to 5 yr
pH stability ^{***}	2–14 short-term 4–13 long-term
Shipping	20% ethanol + 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitation	0.5–1.0 N NaOH
Storage	20% ethanol or 0.1 N NaOH

^{*} 10% breakthrough capacity determined with 4.5 mg/ml human IgG in 40 mM NaOAc, 30 mM NaCl, pH 5.0.

^{**} Elution flow rates <150 cm/hr minimize viscosity-induced backpressure.

^{***} Data derived under accelerated conditions at 60°C.

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 system, Econo™ gradient pump, Model EP-1 Econo pump, and all low-pressure chromatography instruments. They can be connected using the convenient Luer-Lok 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 it clockwise three full turns.

2. 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).

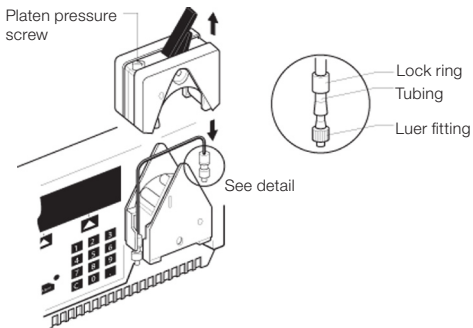


Fig. 1. BioLogic LP system setup.

3. 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 corresponds to the diagram on the valve (Figure 2).

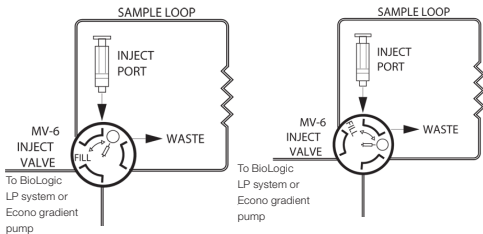


Fig. 2. Connecting to an MV-6 valve.

4. Connect the inlet of the cartridge to the male luer fitting on the MV-6 sample inject valve (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, mount the cartridge vertically, with the arrow on the cartridge pointing down.
5. Connect the outlet to the 1.6 mm ID tubing leading to the BioLogic LP optics module or Econo UV monitor. 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.

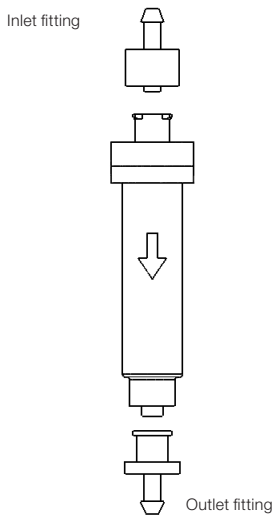


Fig. 3. Column and fittings.

Section 3

Connecting to Other Liquid Chromatography Systems

Bio-Scale™ Mini cartridges can be connected to any liquid chromatography system provided that the pressure limit (3 bar, 45 psi, or 300 kPa) of the cartridges is not exceeded. Set the system pressure limit 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 fittings kits for connecting Bio-Scale Mini cartridges to BioLogic DuoFlow™, HPLC-, or FPLC-type systems.

3.1 BioLogic DuoFlow Systems

The luer to BioLogic system fittings kit (catalog #732-0113) includes ¼–28 female to male and ¼–28 female to female luer fittings to connect one Bio-Scale Mini cartridge to the BioLogic DuoFlow system (Figure 4).



Fig. 4. Components of the luer to BioLogic system fittings kit (catalog #732-0113).

3.2 HPLC Systems

The luer to 10–32 adaptor fittings kit (catalog #732-0112) provides fittings for connecting the cartridge to the nut- and ferrule-type fittings found on many HPLC systems.

Alternatively, connect the cartridge to HPLC systems via a low dead volume, 1/16 in union

with a new piece of stainless steel tubing attached to the union. Slip a short length of 0.8 mm ID tubing over $\frac{1}{16}$ 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 to connect a cartridge to the M6 fittings found on FPLC or related systems.

Alternatively, make a connection using Upchurch Scientific adapters: two $\frac{1}{4}$ -28 to metric adaptors (P-621), one $\frac{1}{4}$ -28 to male luer (P-619), and one $\frac{1}{4}$ -28 to female luer (P-628). Connect the luers to the $\frac{1}{4}$ -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 Nuvia™ cartridges contain 20% ethanol + 0.1 M NaCl (v/v) as the shipping 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 follows:

1. Set the pump flow rate to 1–2 ml/min (240–280 cm/hr) for the 1 ml cartridge or 5–10 ml/min (140–480 cm/hr) for the 5 ml cartridge.
2. Wash the cartridge with degassed low-salt buffer for 2 min.

3. Wash with degassed high-salt buffer for 5 min.
4. Equilibrate 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

Correct 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 concentration. This can be achieved by diluting the sample to the ionic strength of the starting buffer, dialyzing against the starting buffer, or exchange into the starting buffer. Buffer exchange can be accomplished using a number of products (Table 3). The choice of product will depend on sample volume. Filter all samples through a 0.45 μm filter prior to cartridge application.

Table 3. Products for buffer exchange.

Sample Volume	Product	MW Cutoff	Catalog #
50–100 μ l	Bio-Spin [®] 6 columns	6 kD	732-6002
	Bio-Spin 30 columns	30 kD	732-6006
0.1–3 ml	Bio-Scale Mini P6 cartridges	6 kD	732-5304
Up to 3 ml	Econo-Pac [®] 10DG cartridges	6 kD	732-2010
Unlimited	Bio-Gel [®] P-6DG gel	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 μ m filter.

Common buffers are listed in Table 4.

Table 4. Common ion exchange buffers.

Cation	Buffer Range
Acetic acid	4.8–5.2
Citric acid	4.2–5.2
HEPES	6.8–8.2
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
TES	6.8–8.2
Tricine	7.8–8.9

An appropriate starting point for purifying samples is a linear gradient of 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. 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 Purification Scale-Up

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 formats. Nuvia S support is also available in larger amounts, from 25 ml bottles to bulk quantities for scaling-up methods developed using the cartridges. Nuvia S media are fully supported with regulatory support files. In addition, Bio-Rad carries an extensive line of empty chromatography columns for use in laboratory and process scale applications.

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 NaOH, and 6 M guanidine hydrochloride.

1. Sanitize the support in the column with 2–4 bed volumes of 1.0 N NaOH at 50–100 cm/hr while maintaining a contact time of at least 40 min.

2. To reequilibrate the column, wash it with 2–4 bed volumes of 0.5–2 M NaCl solution (containing 50–100 mM buffer).
3. If lipid removal is required, wash the column with a 20–70% 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, stored in 20% ethanol solution, and capped for extended storage.

Section 6

Ordering Information

Bio-Scale™ Mini Cartridges

For a list of available cartridges*, visit www.bio-rad.com/cartridges/.

Description	1 x 1 ml	5 x 1 ml	1 x 5 ml	5 x 5 ml
Nuvia™ S	732-4420	732-4421	732-4422	732-4423
UNOsphere™ Q	----	732-4100	731-4102	731-4104
UNOsphere S	----	732-4110	731-4112	731-4114
UNOsphere Rapid S	----	732-4400	732-4401	732-4402
UNOsphere SUPrA™	732-4200	732-4201	732-4202	----
Macro-Prep® High Q	----	732-4120	732-4122	732-4124
Macro-Prep High S	----	732-4130	732-4132	732-4134
Macro-Prep DEAE	----	732-4140	732-4142	732-4144
CHT™ Type I	----	----	732-4322	732-4324
CHT Type II	----	----	732-4332	732-4334
CFT™ Type II	----	----	732-4405	732-4406
Bio-Gel® P-6	----	----	732-4502	732-4504
Affi-Prep® protein A	----	732-4600	732-4602	----
Profinity™ IMAC	----	732-4610	732-4612	732-4614
DEAE Affi-Gel® Blue	----	----	732-4632	732-4634
Affi-Gel Blue	----	----	732-4642	732-4644

* Media are available in volumes 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 1 cartridge 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 1 cartridge to the BioLogic DuoFlow™ system

- 731-8226 **3.2 mm Barb to Male Luer,**
pkg of 25
- 731-8225 **1.6 mm Barb to Male Luer,**
pkg of 25
- 731-8224 **0.8 mm Barb to Male Luer,**
pkg of 25
- 731-8223 **3.2 mm Barb to Female Luer,**
fits inlet and outlet of Econo-
Column chromatography
columns and low-pressure
tubing, pkg of 25
- 731-8222 **1.6 mm Barb to Female Luer,**
pkg of 25
- 731-8221 **0.8 mm Barb to Female Luer,**
pkg of 25

Section 7

Related Reading

Harris ELV and Angal S (1989). Protein Purification Methods: A Practical Approach (Oxford: IRL Press).

Scopes RK (1987). Protein Purification: Principles and Practice (Second Edition) (New York: Springer-Verlag).

Snyder LR and Kirkland JJ (1979). Introduction to Modern Liquid Chromatography (Second Edition) (New York: Wiley).

Gagnon P (1997). Avoiding Instrument-Associated Aberrations in Purification Scale-Up and Scale-Down, BioPharm 10, 42–45.



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