Bio-Scale™ Mini CHT™ Ceramic Hydroxyapatite Cartridges, 5 ml

Instruction Manual

Catalog # 732-4322
732-4324
732-4332
732-4334

BIO-RAD

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Section 1
Introduction

Bio-Scale™ Mini cartridges are easy-to-use, prepacked chromatographic cartridges for fast, reproducible chromatographic separations. They have a 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 (page 22) 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 CHT cartridges are packed with Type I or Type II ceramic hydroxyapatite supports. These supports are based on hydroxyapatite, a form of calcium phosphate used in chromatographic separations of biomolecules. CHT ceramic hydroxyapatite is a spherical, macroporous form of hydroxyapatite. Unlike most other chromotagraphic absorbents, CHT is both the ligand and the support matrix. CHT Type I has a higher protein binding capacity and greater capacity for acidic proteins. CHT Type II has a lower protein binding capacity but provides better resolution of nucleic acids and certain other proteins. The Type II material also has a very low affinity for albumin and is especially suitable for the purification of many species types and classes of immunoglobulins. Applications of hydroxyapatite chromatography include the purification of different subclasses of monoclonal and polyclonal antibodies, antibodies that differ in light chain composition, antibody fragments, isozymes, supercoiled DNA from linear duplexes, and single-stranded from double stranded DNA. Detailed product information is given in Tables 1 and 2.

Table 1: Bio-Scale Mini CHT Ceramic Hydroxyapatite Cartridge Specifications

Size	5 ml bed volumes
Dimension	40 mm length x 12.6 mm inner diameter
Maximum pressure tolerance	45 psi
Recommended flow rate	5–10 ml/min (140–481 cm/hr)
Maximum flow rate	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	Dry
Storage recommendation	0.1 N NaOH
Autoclavability	Not autoclavable

Table 2. Product Description

	Type I	Type II
Functional groups	Ca^{2+}, PO_4, OH	Ca^{2+}, PO_4, OH
Observed dynamic binding capacity		
lysozyme (Lys)	≥ 25 mg Lys/g CHT	≥ 12.5 mg Lys/g CHT
Nominal pore diameter	600–800 Å	800–1,000 Å
Maximum backpressure	100 bar (1,500 psi)	100 bar (1,500 psi)
Nominal mean particle size	20 ± 2, 40 ± 4, and 80 ± 8 µm	
Bulk density	0.63 g/ml	0.63 g/ml
Observed dynamic binding capacity		
IgG	25–60 mg IgG/ml CHT*	15–25 mg IgG/ml CHT*
Typical linear flow rate range	50–1,000 cm/hr	
pH stability	6.5–14	
Base stability	at least 21 months in 1 N NaOH	
Regeneration	500 mM sodium phosphate, pH 7 1,000 mM trisodium phosphate, pH 11–12	
Autoclavability (bulk)	121°C, 20 min in phosphate buffer, pH 7	
Sanitization	1–2 N NaOH	
Recommended column storage	0.1 N NaOH	
Shelf life (dry, unused material)	85 months stored dry, sealed, and at room temperature	

* 40 µm particles, 300 cm/hr, 5 mM sodium phosphate, pH 6.5

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.)

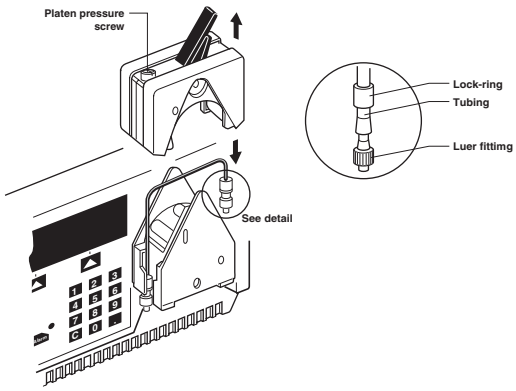

Fig. 1. Biologic LP setup.

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 correspond to the printed diagram on the valve. (Figure 2).

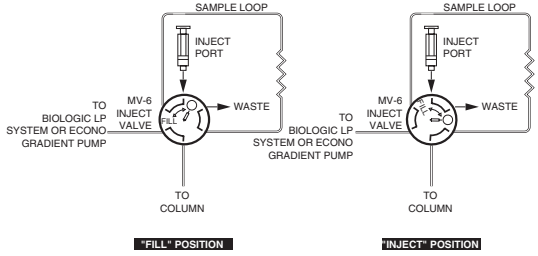

Fig. 2. Connecting to a MV-6 Valve.

3. Hold the cartridge vertically with outlet in the downward direction. 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, 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, and then connect to the bottom of the male luer on the Bio-Scale Mini cartridge.

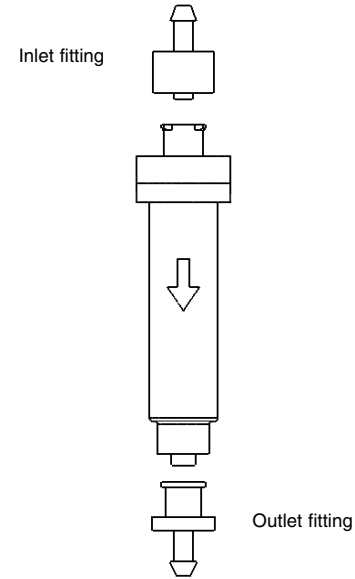

Fig. 3. Column and Fittings.

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 fitting 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 number 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 number 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 number 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 the following Upchurch Scientific® Quick Connect Luer Adaptors: two Upchurch P-621 adaptor, 1/4–28, to metric adaptors, one Upchurch P-619 adaptor, 1/4–28, to male luer, and one Upchurch P-628 adaptor, 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 CHT ceramic hydroxyapatite cartridges are shipped dry. Prior to use, the following cartridge conditioning steps should be followed:

1. Hold the cartridge vertically with the outlet in the downward direction and then tap it against the surface of the bench about 10 times.
2. Remove the luer caps and then attach to your chromatography system with the outlet pointed down (arrow pointing down).
3. It is recommended that the cartridge be equilibrated with 25 ml of 1 N NaOH at 150 cm/hr (2 ml/min).
4. Equilibrate the cartridge at 150 cm/hr (2 ml/min) with 25–50 ml of 100 to 400 mM sodium phosphate buffer, between pH 6.5 and pH 8.0.

5. Equilibrate the cartridge with running buffer and operatate the cartridge according to the CHT instruction manual or according to your protocol.

4.1 Sample Preparation

Proper 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'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 3. 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

Hydroxyapatite chromatography is usually performed using increasing linear salt 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. In order to promote the stability of CHT, buffers are recommended to include low concentrations of either phosphate or

calcium. Common buffers for hydroxyapatite chromatography are listed in Table 4.

An appropriate starting point for purifying samples is a linear gradient from 5–500 mM sodium phosphate pH 6.8, spanning 10 to 20 column volumes at 2.0 ml/min for the 5 ml cartridge. For separations of monoclonal antibodies from aggregates, an alternative purification protocol is eluting with a linear gradient from 0-2 M sodium chloride in 5-20 mM sodium phosphate, pH 6.5. The separation can be optimized by changing the gradient profile. At the end of each run the cartridge can be regenerated with 500 mM potassium or sodium phosphate buffer, neutral pH; or 400 mM trisodium phosphate, pH 11–12; 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. CHT ceramic

hydroxyapatite media is available in larger amounts, from 10 g bottles to bulk quantities, for scaling up methods developed for using the cartridges. CHT ceramic hydroxyapatite is fully supported by Regulatory Support Files. In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory scale to process scale.

Table 4. CHT stability in various buffers.*

		CHT Suitability	
pH	**Buffer**	**10 cycles**	**50 cycles**
5.0	Acetate + 5 mM PO_4	—	n/a
5.5	Acetate + 5 mM PO_4	-	—
6.0	Acetate + 5 mM PO_4	+	-
6.0	Succinate + 5 mM PO_4	-	—
6.5	Succinate + 5 mM PO_4	+/-	+/-
6.5	Acetate + 5 mM PO_4	+	n/a
6.5	Phosphate (5 mM)	+	n/a
6.5	Acetate + 5 mM PO_4	+	+
6.5	MES + 5 mM PO_4	+	+
6.5	Imidazole + 5 mM PO_4	+	+/-
6.5	Glycine + 5 mM PO_4	+	+
6.5	Arginine + 5 mM PO_4	+	+
6.5	Tris + 5 mM PO_4	+	n/a
7.0	Phosphate (5 mM)	+	+

+	No statically significant mass loss observed
+/-	Slight (0–1%) loss
-	Small (1–2%) loss
—	Significant (>2%) loss
n/a	Not applicable

		CHT Suitability	
pH	Buffer	10 cycles	50 cycles
7.0	MES + 5 mM PO_4	+	n/a
7.0	Acetate + 5 mM PO_4	+	n/a
7.0	Imidazole + 5 mM PO_4	+	+
7.0	Glycine + 5 mM PO_4	+	n/a
7.0	Arginine + 5 mM PO_4	+	n/a
7.0	HEPES + 5 mM PO_4	+	n/a
7.0	Tris + 5 mM PO_4	+	n/a
7.5	Phosphate (5 mM)	+	n/a
7.5	MES + 5 mM PO_4	+	n/a
7.5	Imidazole + 5 mM PO_4	+	+
7.5	Acetate + 5 mM PO_4	+	n/a
7.5	HEPES + 2 mM PO_4	+	n/a
7.5	HEPES + 5 mM PO_4	+	n/a
7.5	Tris + 2 mM PO_4	+	n/a
7.5	Tris + 5 mM PO_4	+	+
8.5	Tris + 5 mM PO_4	+	n/a

* All experiments performed in small scale columns. Each cycle used approximately 35 column volumes of buffer to simulate an equilibration and long gradient, as well as five column volumes of 1 N NaOH to simulate regeneration.

Section 5
Care of the Cartridge

5.1 Cleaning

After repeated use, a CHT cartridge may require thorough cleaning and regeneration to remove bound contaminants. Most bound contaminants may be removed by the following protocol:

Step 1. Wash the cartridge with 3–5 column volume (5 ml/min for the 5 ml) of 500 mM potassium or sodium phosphate, then 5 column volumes of starting buffer.

Step 2. Equilibrate the cartridge with at least 5 column volumes (5 ml/min for the 5 ml) of starting buffer. If bound contaminants persist after following the steps above, use one of the alternative procedures listed in wash alternatives.

5.2 Wash Alternatives

Perform wash alternatives with any of the following alternative buffers in place of step 1. Continue with step 2 listed in Section 5.1.

- 1–2 M KCl or NaCl
- 6 M urea
- 8 M guanidine-HCl

All the wash alternative buffers should contain 5 mM phosphate at neutral pH.

5.3 Autoclaving

Bio-Scale Mini cartridges are not autoclavable.

5.4 Storage

After the cartridges are washed with deionized water, Bio-Scale Mini CHT cartridges should be purged and stored with 0.1 N NaOH. Higher concentrations of NaOH may be used if desired. Used CHT, after being regenerated and sanitized, can be stored in up to 1.0 N NaOH at room temperature.

Section 6
Technical Support

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

Section 7
Ordering Information

Prepacked Bio-Scale Mini Cartridges*

Description	5 x 1 ml	1 x 5 ml	5 x 5 ml
CHT Ceramic Hydroxyapatite, Type I, 40 µm		732-4322	732-4324
CHT Ceramic Hydroxyapatite, Type II, 40 µm		732-4332	732-4334
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/

** Larger package sizes of media are available for process scale chromatography. Please contact 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/ 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

P. Gagnon et al., A Ceramic Hydroxyapatite-Based Purification Platform, BioProcess International 4 (2), 50–60 (2006)

T. Ogawa et al., Effect of pH on Gradient Elution of Proteins on Two Types of Macro-Prep Ceramic Hydroxapatite, Prep Tech ’95, Industrial Separation Science Conference, East Rutherford, NJ (1995)

S.R. Shepard et al., Discoloration of ceramic hydroxyapatite used for protein chromatography, J Chromatography A 891, 93–98 (2000)

E. Dolinski et al., Purification of a Fusion Protein by Ceramic Hydroxyapatite Chromatography, Second International Conferences on Hydroxyapatite and Related Products, San Francisco, Ca (2001)

M. Gorbunoff et al., The Interaction of Proteins with Hydroxyapatite I: Role of Protein Charge and Structure, Analytical Biochemistry136, 425–432 (1984)

M. Gorbunoff et al., The Interaction of Proteins with Hydroxyapatite II: Role of Acidic and Basic Groups, Analytical Biochemistry 136, 433–439 (1984)

M. Gorbunoff et al., The Interaction of Proteins with Hydroxyapatite III: Mechanism., Analytical Biochemistry 136, 440–445 (1984)

D. J. Josic et al., Purification of monoclonal antibodies by hydroxyapatite HPLC and size exclusion HPLC, Hoppe-Seylars Zeitschrift fur Physiologische Chemie 372, 149–156 (1991)

T. Kawasaki et al., Hydroxyapatite as a Liquid Chromatographic Packing. J. Chromatography 544, 147–184 (1991)

L. Cummings et al., Ceramic Hydroxyapatite Offers a New, Old Chromatography Application Tool, Genetic and Engineering News 14 (1994)

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