

# Bio-Scale Ceramic Hydroxyapatite Type I Columns



## Introduction

Bio-Scale prepacked ceramic hydroxyapatite, type I (CHT-I) columns meet the needs of the bio-chromatographer for rapid and reproducible high resolution separations of biomolecules including proteins, peptides, and polynucleotides.

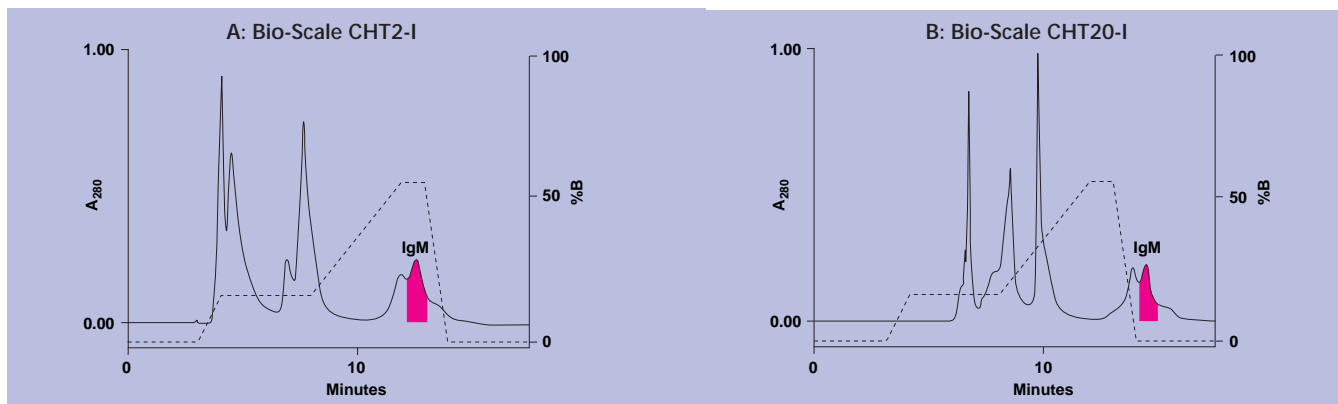
- Biocompatible materials preserve the protein's integrity
- Four sizes available (2, 5, 10, and 20 ml) for easy scale-up
- Fingertight fittings eliminate the need for tools
- Adjustable bed support minimizes column voids

## Convenience

The Bio-Scale column's unique design offers all the convenience needed for working with medium or high pressure systems.

## Scale-up

The availability of four column sizes (bed volumes of 2, 5, 10, and 20 ml) provides unrivaled flexibility for the economical and predictable scale up of separation and purification protocols without sacrificing resolution due to overloading (see Figure 1).



**Fig. 1. IgM purification.** Scale-up from 250  $\mu$ l to 2.3 ml of mouse ascites (2:1 dilution) using the Bio-Scale CHT-I columns. **A:** Bio-Scale CHT2-I, Sample: 250  $\mu$ l, Buffer A: 10 mM NaPB, pH 6.8, Buffer B: 500 mM NaPB, pH 6.8, Flow rate: 1 ml/min. **B:** Bio-Scale CHT20-I, Sample: 2.3 ml, Flow rate: 4.6 ml/min.

## ■ The CHT Separation Medium

Each Bio-Scale CHT-I column contains the newly developed, chemically pure form of hydroxyapatite. The spherical, ceramic nature of the 10 µm Macro-Prep® CHT support overcomes the physical and chemical instability that limited many applications of traditional crystalline forms of hydroxyapatite for high resolution chromatography. The narrow particle size distribution and an optimized packing procedure produce excellent resolution of biomolecules at high flow rates and with very low backpressures.

The CHT support demonstrates high affinity for basic proteins of relatively high pI and lower affinity for proteins of relatively low pI. The hydroxyapatite structure (Ca<sup>2+</sup> ions at the positively charged centers and PO<sub>4</sub><sup>3-</sup> at the negatively charged centers) results in essentially a mixed-mode ion exchange separation. Separation of biomolecules using hydroxyapatite is a very useful complement to other separation techniques such as ion exchange and hydrophobic interaction chromatography, particularly in the later stage of a purification scheme. It often resolves components that other techniques fail to separate. The support binds proteins under mild conditions using neutral phosphate buffer systems. Selective elution is accomplished with higher concentrations of phosphate and/or a pH gradient. Recovery of protein catalytic activity is normally high due to the gentle elution conditions employed.

## ■ Stability of the Macro-Prep CHT-I Support

The columns are stable over the pH range 5.5–14, allowing easy cleaning and regeneration. The Macro-Prep support is compatible with aqueous solutions of 6 M guanidine-HCl and

8 M urea. Detergents such as Triton®, CHAPS, CTAB, and 1% SDS, and organic solvents such as methanol, ethanol, and isopropanol may also be used.

Buffers containing calcium chelators such as EDTA and EGTA should be avoided. If chelating agents must be present in the buffers, the addition of sodium chloride may be necessary.

**Table 1. Properties of Macro-Prep 10 CHT-I Support**

Type of exchanger	Hydroxyapatite
Functional group	Ca <sup>2+</sup> , PO <sub>4</sub> <sup>-3</sup>
Dynamic binding capacity	> 25 mg Lysozyme/g CHT-I
Average particle size (µm)	10 ± 2.0
pH stability	5.5–14

## ■ Top-Off Resin

When the top of the resin bed becomes fouled and conventional hygiene steps do not restore performance, then a few mm of the bed can be removed and replaced with fresh resin obtainable from Bio-Rad.

## ■ The Macro-Prep CHT-I Family

For low pressure laboratory scale protein purification, the Macro-Prep CHT-I support is also available in a 20, 40, and 80 µm particle sizes. This support is available in 100 g and 500 g bottles, and in bulk for scale-up and process scale applications. All of the Macro-Prep supports are produced under GMP and have Drug Master Files. For more information, contact your local Bio-Rad office.

## Ordering Information

Catalog Number	Column Name	Column Dimension	Bed Volume	Recommended Protein Loading	Recommended Flow Rate (ml/min)	Maximum Operating Pressure
751-0021	Bio-Scale CHT2-I Column	7 x 52 mm	2 ml	20 mg	0.5–3.0	1,000 psi
751-0023	Bio-Scale CHT5-I Column	10 x 64 mm	5 ml	50 mg	0.5–5.0	750 psi
751-0025	Bio-Scale CHT10-I Column	12 x 88 mm	10 ml	100 mg	0.5–7.0	600 psi
751-0027	Bio-Scale CHT20-I Column	15 x 113 mm	20 ml	200 mg	0.5–10.0	500 psi
751-0029	Top-off Resin Kit, CHT-I, 1 ml					

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**BIO-RAD**

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