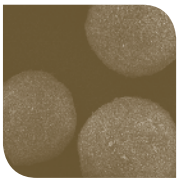

CHROMATOGRAPHY

MPC™ Ceramic Hydroxyfluoroapatite

- High physical and chemical stability
- Unmatched selectivity
- Clearance of impurities and aggregates in a single step
- Rapid and simple column packing

Separation of Impurities in a Single Step

The newest addition to Bio-Rad's line of ceramic apatite chromatography media is MPC ceramic hydroxyfluoroapatite. MPC is a second-generation CHT™ ceramic hydroxyapatite mixed-mode chromatography media, a composite of hydroxyapatite and fluoroapatite prepared by chemically substituting 25% of the hydroxyl groups of hydroxyapatite nanocrystals with a fluorine reagent. Its chemical formula is $Ca_{10}(PO_4)_6(OH)_{1.5}(F)_{0.5}$. The partial fluorination confers a more chemically stable form of the matrix during pH excursions that are inherent to buffer exchanges. MPC has unique separation properties, unmatched selectivity, and resolution similar to CHT, including binding capacity (Tables 1 and 2), protein separations (Figure 1), and clearance (Figures 2 and 3, Table 3). MPC is 40 µm and is directly comparable to CHT Type I, 40 µm (Tables 1 and 2).

Mechanism of Action and Standard Chromatography

MPC interacts with biomolecules by multiple modes. Cation exchange occurs when negatively charged phosphate groups interact with protein amino groups. Much stronger coordination complexes can form between carboxyl clusters, phosphoryl moieties, or both on biomolecules and the calcium sites on MPC via metal affinity. Repulsion effects and the geometric charge distribution on MPC provide unique selectivity. Typically, acidic, basic, and neutral proteins are bound to MPC using low ionic strength phosphate buffer. Elution is accomplished through the use of a sodium chloride or phosphate gradient of increasing strength or via similar step elutions. Regeneration of the support with phosphate buffers at neutral pH is followed by sanitization with up to 2 N NaOH. Regardless of greater chemical stability, we recommend implementing pH mitigation steps, for example, the surface neutralization system (SNS). A pilot-scale study with MPC using SNS achieved 125 cycles. For more detailed information, refer to the CHT user's guide at **www.bio-rad.com/CHTGuide**.

Table 1. Specifications.

	CHT Type I, 40 µm	MPC
Functional groups	Ca^{2+}, PO_4, OH	Ca^{2+}, PO_4, OH, F
Observed dynamic binding capacity lysozyme (Lys)	≥25 mg Lys/g CHT	≥25 mg Lys/g MPC
Nominal pore diameter	600–800 Å	600–800 Å
Maximum backpressure	100 bar (1,500 psi)	100 bar (1,500 psi)
Nominal mean particle size	40 ± 4 µm	40 ± 4 µm
Tap settled density* (g/ml tap settled bed)	0.63 g/ml	0.72 g/ml

* Under ideal conditions.

BIO-RAD

Table 2. Characteristics.

	CHT Type I, 40 µm	MPC
Observed dynamic binding capacity IgG	25–60 mg IgG/ml CHT*	25–50 mg IgG/ml MPC**
Typical linear flow rate range	50–1,000 cm/hr	50–1,000 cm/hr
pH stability	6.5–14 pH	6.5–14 pH
Base stability	At least 1 year in 0.1 N NaOH	At least 1 year in 0.1 N NaOH
Regeneration	0.4–0.5 M sodium phosphate, pH 7–7.5, is generally sufficient. If higher concentrations are needed, use potassium phosphate	0.4–0.5 M sodium phosphate, pH 7–7.5, is generally sufficient. If higher concentrations are needed, use potassium phosphate
Autoclavability (bulk)	121°C, 20 min in phosphate buffer, pH 7	121°C, 20 min in phosphate buffer, pH 7
Sanitization	1–2 N NaOH	1–2 N NaOH
Recommended column storage	0.1 N NaOH	0.1 N NaOH

* 40 µm particles, 300 cm/hr, 5 mM sodium phosphate, pH 6.5.
** 40 µm particles, 300 cm/hr, 5 mM sodium phosphate, 25 mM NaCl, pH 6.5.

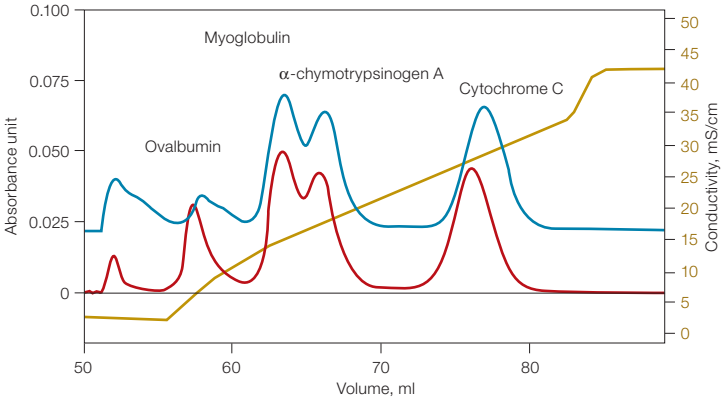

Fig. 1. Separation of protein standards. MPC separates the standard protein mixture similarly to CHT Type I, 40 µm. Sample: 6 mg of ovalbumin, 3 mg of myoglobulin, 3 mg of α-chymotrypsinogen A, and 3 mg of cytochrome C in 1.5 ml 10 mM sodium phosphate (NaPi), pH 6.8. CHT column: 0.5 x 10.3 cm, packed bed volume 2 ml; MPC column: 0.5 x 10.4 cm, packed bed volume 2 ml. Protocol: 1.55 ml/min; 400 mM NaPi, pH 6.8, 5 column volumes (CV); 10 mM NaPi, pH 6.8, 15 CV; load volume 50 µl; linear gradient elution 0 to 75% 400 mM NaPi, pH 6.8, over 15 CV; strip 400 mM NaPi, pH 6.8, 5 CV; sanitization 1 N NaOH, 5 CV. CHT (—); MPC (—).

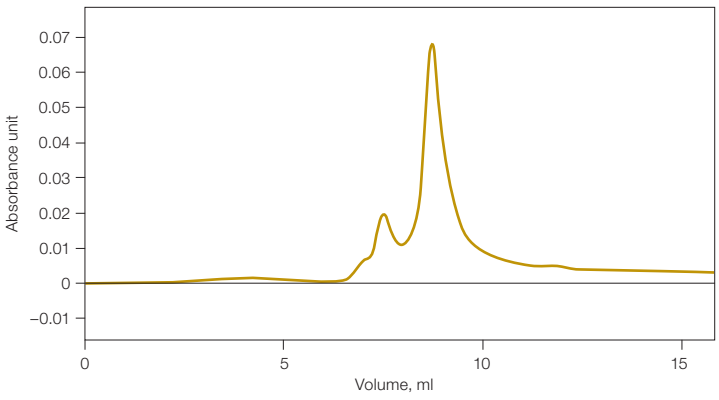

Fig. 2a. MAb-S size exclusion chromatography (SEC) profile of starting material. SEC profile shows the higher molecular weight impurities (dimers/aggregates) and monomers in the starting material (material applied to the second step (Figure 2b) using CHT or MPC). Sample: 2.9 mg/ml mAb-S in 10 mM NaPi, pH 7. Column: 300 x 7.8 mm, Bio-Sil® SEC 250 HPLC. Protocol: 1 ml/min; equilibration in 1 ml of 0.1 M NaPi, 0.15 M NaCl, 0.02% azide, pH 7; injection volume 0.1 ml; elution in 15 ml of 0.1 M NaPi, 0.15 M NaCl, 0.02% azide, pH 7.

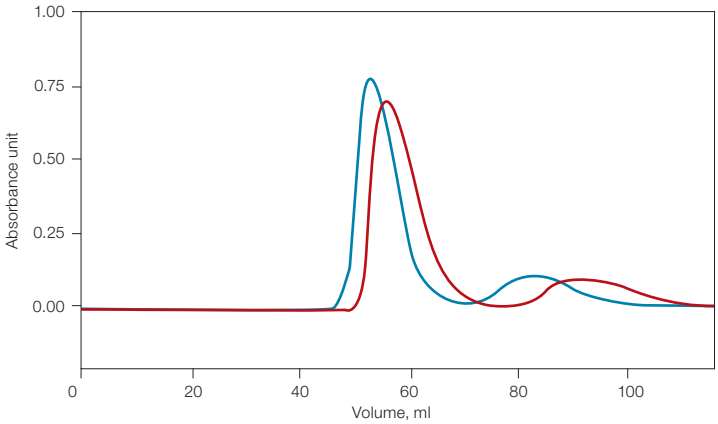

Fig. 2b. MAb-S purification profile. Elution profile shows separation of the monomer from higher molecular weight impurities. Sample: 7.26 mg mAb-S/ml packed bed in 5 ml 10 mM NaPi, pH 7. Column: 0.5 x 10 cm, packed bed volume 2.1 ml of UNOsphere SUPrA™ rProtein A media. Protocol: 0.5 ml/min; 10 mM NaPi, pH 7, 5 column volumes (CV); load volume 5 ml; 10 mM NaPi, pH 7, 5 CV; linear gradient elution 0 to 100% 10 mM NaPi, 1 M NaCl, pH 7, over 40 CV; strip 10 mM NaPi, 1 M NaCl, pH 7, 5 CV; sanitization 1 N NaOH, 5 CV. CHT (—); MPC (—).

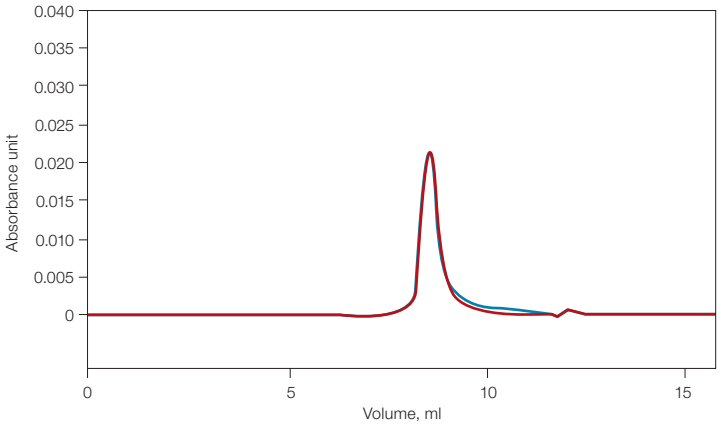

Fig. 2c. SEC profile of pooled monomer fractions. The SEC profile of the pooled fractions confirms aggregate clearance from the monomer. Column: 300 x 7.8 mm, Bio-Sil SEC 250 HPLC. Protocol: 1 ml/min; 100 mM NaPi, 150 mM NaCl, 0.02% azide, pH 7, 1 ml; load volume 0.1 ml; elution 100 mM NaPi, 150 mM NaCl, 0.02% azide, pH 7, 15 ml. CHT (—); MPC (—).

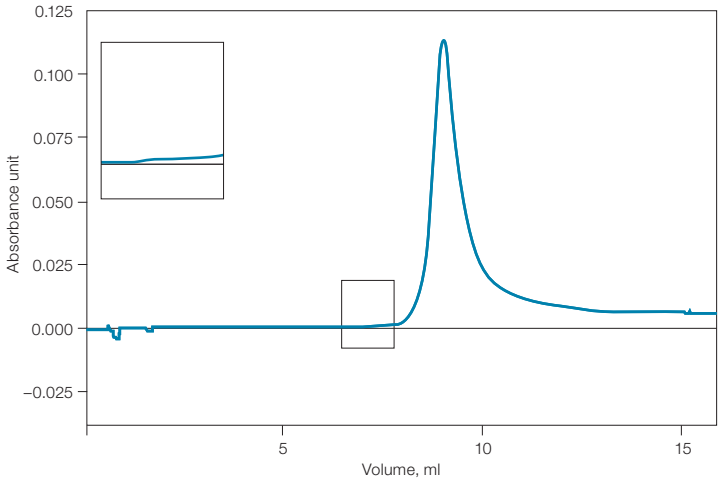

Fig. 3a. MAb-G SEC profile of starting material. SEC profile shows the higher molecular weight impurities (dimers/aggregates) and monomers in the starting material (material applied to the second step (Figure 3b) using CHT or MPC). Sample: 4.41 mg/ml mAb-S in 10 mM NaPi, pH 7. Column: 300 x 7.8 mm, Bio-Sil SEC 250 HPLC. Protocol: 1 ml/min; equilibration in 1 ml of 0.1 M NaPi, 0.15 M NaCl, 0.02% azide, pH 7; injection volume 0.1 ml; elution in 15 ml of 0.1 M NaPi, 0.15 M NaCl, 0.02% azide, pH 7.

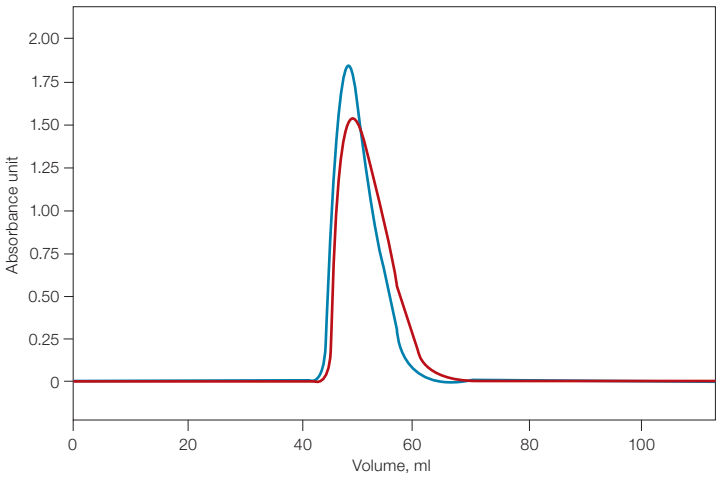

Fig. 3b. MAb-G purification profile. Elution profile shows separation of the monomer from higher molecular weight impurities. Protocol as described in Figure 2b. CHT (—); MPC (—).

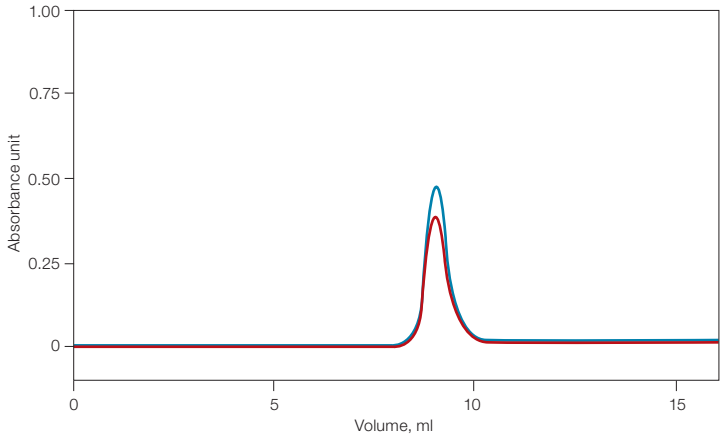

Fig. 3c. SEC profile of pooled monomer fractions. The SEC profile of the pooled fractions confirms aggregate clearance from the monomer. Protocol as described in Figure 2c. CHT (—); MPC (—).

Table 3. Quantified data from all assays. All pooled fractions and mAb starting material purified on MPC and CHT were analyzed for host cell protein (HCP ELISA kit, Cygnus Technologies), for dsDNA (PicoGreen dsDNA quantitation kit, Invitrogen Corporation), for protein A leachables (Protein A ELISA kit, Cygnus Technologies), and for aggregate clearance by SEC (Bio-Sil SEC 250, Bio-Rad Laboratories, Inc).

Sample	IgG Concentration, mg/ml	Protein A, ppm	HCP, ppm	DNA, ppm	Dimer/Aggregate, %
MAb-S					
Protein A eluate	2.90	33	24	21	21.62
CHT pool	0.40	<0.6	<2	2.6	<0.03
MPC pool	0.46	<0.5	<2	2.8	<0.03
MAb-G					
Protein A eluate	4.41	17	<2	0.82	4.39
CHT pool	0.91	<0.3	<1	1.4	<0.03
MPC pool	0.77	<0.3	<1	1.4	<0.03

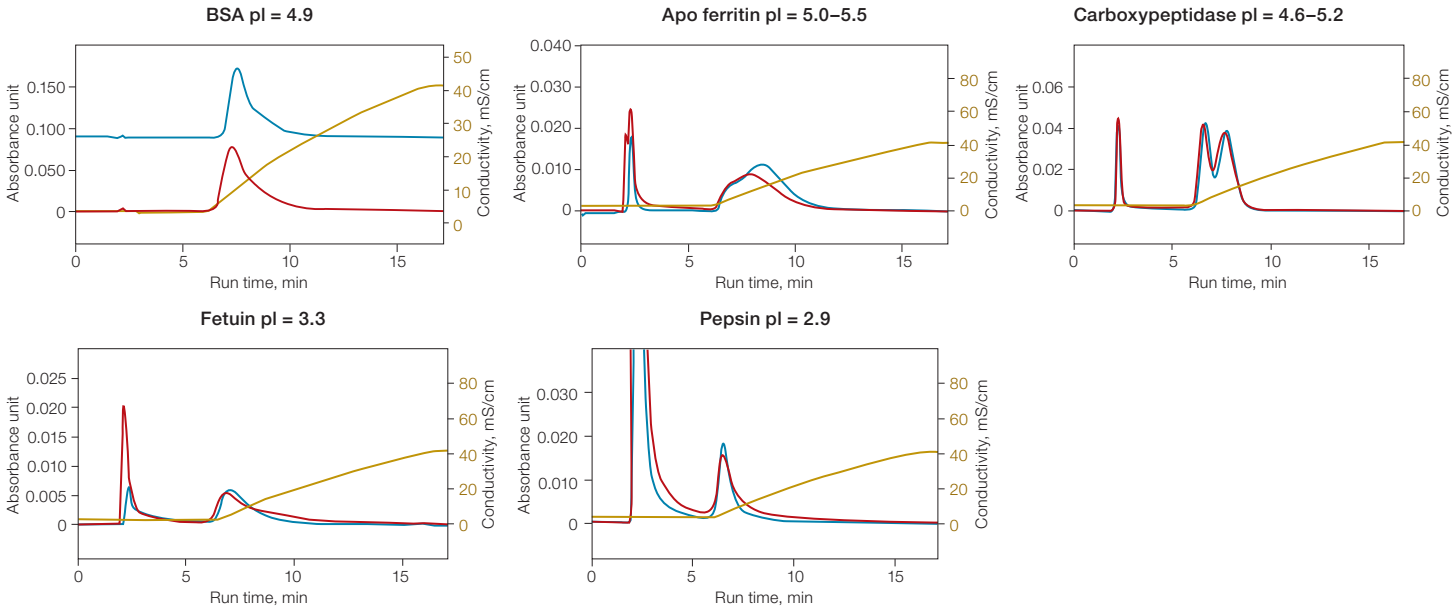

Fig. 4. Elution profiles of five acidic proteins. CHT and MPC produce similar elution characteristics for the five acidic proteins studied. Samples: porcine carboxypeptidase B (CBP), pI 4.6–5.2, pepsin pI 2.9, fetuin, pI 3.3, BSA, pI 4.9, and apoferritin, pI 5.0–5.5, each prepared to a final concentration of 2–3 mg/ml in water. Column: 0.7 x 2.6 cm, packed bed volume 1 ml, executed using a BioLogic DuoFlow™ system and software. Protocol: 300 cm/hr; load volume 100 µl; linear gradient elution 0 to 100% 0.02 M HEPES, 0.01 M NaPi, pH 6.5, to 0.02 M HEPES, 0.4 M NaPi, pH 6.5, over 20 CV. CHT (—); MPC (—).

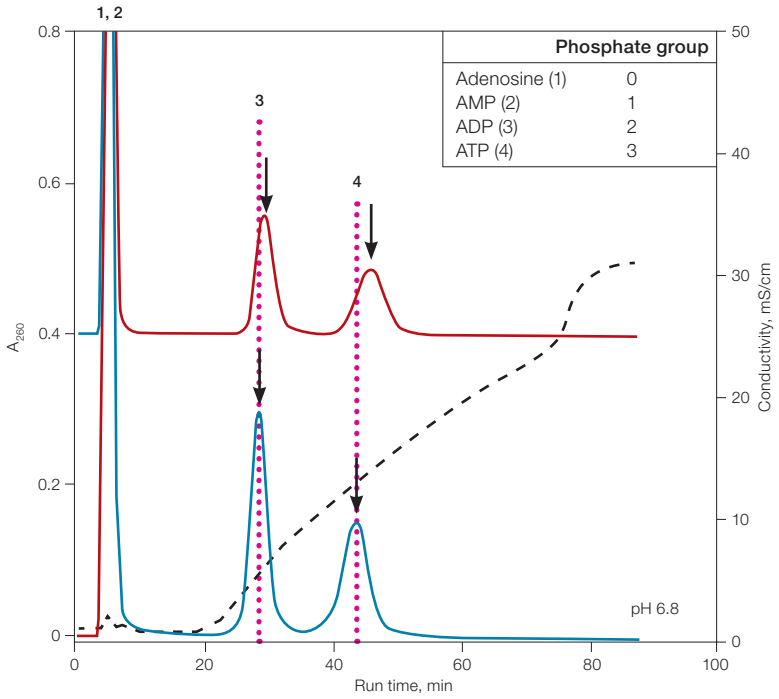

Fig. 5. Nucleic acids separation profile. Elution profile shows separation of ADP and ATP. Sample: 1 mg each of adenosine, AMP, ADP, and ATP mixture dissolved in 1 ml of 10 mM NaPi, pH 6.8, filtered with 0.22 µm membrane. Column: 0.4 x 10 cm. Protocol: 1 ml/min; load volume 0.03 ml; wash 10 mM NaPi, pH 6.8, 2 CV; linear gradient elution 0 to 75% 400 mM NaPi, pH 6.8, over 12 CV; strip 400 mM NaPi, pH 6.8, 4 CV. CHT (—); MPC (—).

Storage

MPC ceramic hydroxyfluoroapatite should be stored in 0.1 N NaOH at room temperature. In dry powder form, MPC ceramic hydroxyfluoroapatite should be stored in a secured, closed container at room temperature.

Technical Assistance

For more detailed information on process step development, use recommended steps as described in the CHT Applications Guide (**www.bio-rad.com/CHTGuide**). Regulatory support file is available upon request. Bio-Rad Laboratories is an ISO 9001 registered corporation. For additional information and technical assistance, contact your local Bio-Rad office. In the USA and Canada, call 1-800-4BIORAD. You can also send an email to lsg_techserv_us@bio-rad.com. Visit us on the Web at **www.bio-rad.com** for more information on Bio-Rad's complete line of process chromatography supports.

Ordering Information

Catalog #	Description
MPC Ceramic Hydroxyfluoroapatite, Type I	
158-0200	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 10 g
157-0200	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 100 g
157-0201	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 1 kg
157-0205	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 5 kg
Foresight™ Columns	
732-4737	**Foresight™ MPC™ Type I Column**, 40 µm, 1 ml
732-4757	**Foresight MPC Type I Column**, 40 µm, 5 ml
Foresight Plates*	
732-4785	**Foresight MPC Type I Plates**, 40 µm, 20 µl
Foresight RoboColumn Units**	
732-4828	**Foresight MPC Type I RoboColumn Units**, 40 µm, 200 µl
732-4829	**Foresight MPC Type I RoboColumn Units**, 40 µm, 600 µl

* Package size: 2 x 96-well plates

** Package size: one row of eight columns

Related Items

Catalog #	Description
CHT Ceramic Hydroxyapatite, Type I	
158-2000	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 10 g
157-0020	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 100 g
157-0021	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 1 kg
157-0025	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 5 kg
158-4000	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 10 g
157-0040	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 100 g
157-0041	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 1 kg
157-0045	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 5 kg
158-8000	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 10 g
157-0080	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 100 g
157-0081	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 1 kg
157-0085	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 5 kg

Catalog #	Description
CHT Ceramic Hydroxyapatite, Type II	
158-2200	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 10 g
157-2000	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 100 g
157-2100	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 1 kg
157-2500	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 5 kg
158-4200	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 10 g
157-4000	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 100 g
157-4100	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 1 kg
157-4500	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 5 kg
158-8200	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 10 g
157-8000	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 100 g
157-8100	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 1 kg
157-8500	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 5 kg
Foresight Columns	
732-4735	**Foresight™ CHT™ Type I Column**, 40 µm, 1 ml
732-4755	**Foresight CHT Type I Column**, 40 µm, 5 ml
732-4736	**Foresight CHT Type II Column**, 40 µm, 1 ml
732-4756	**Foresight CHT Type II Column**, 40 µm, 5 ml
Foresight Plates*	
732-4716	**Foresight CHT Type I Plates**, 40 µm, 20 µl
732-4718	**Foresight CHT Type II Plates**, 40 µm, 20 µl
Foresight RoboColumn Units**	
732-4822	**Foresight CHT Type I RoboColumn Units**, 40 µm, 200 µl
732-4823	**Foresight CHT Type I RoboColumn Units**, 40 µm, 600 µl
732-4825	**Foresight CHT Type II RoboColumn Units**, 40 µm, 200 µl
732-4826	**Foresight CHT Type II RoboColumn Units**, 40 µm, 600 µl

* Package size: 2 x 96-well plates

** Package size: one row of eight columns

For More Information

Request or download bulletins 6086 and 5667

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