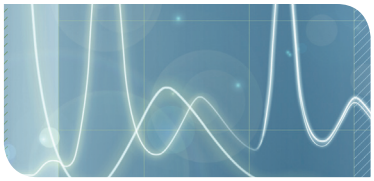

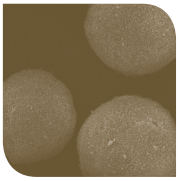

CHROMATOGRAPHY

CHT™ Ceramic Hydroxyapatite

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

A Matrix with Unique Separation Properties and Unparalleled Selectivity and Resolution

CHT ceramic hydroxyapatite is a spherical, macroporous form of hydroxyapatite. The ceramic material overcomes many of the limitations of traditional crystalline hydroxyapatite and provides the throughput, stability, and reproducibility required for industrial biopharmaceutical manufacturing. It has unique separation properties and unparalleled selectivity and resolution.

CHT ceramic hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a chemically pure form of hydroxyapatite that has been sintered at high temperatures to yield a physically and chemically robust support. Often, it will separate proteins shown to be homogenous by electrophoretic and other chromatographic techniques. Due to its consistently reproducible results over many cycles at high flow rates, CHT ceramic hydroxyapatite is ideal for large-scale bioprocess applications. Applications include the purification of isoproteins, antibody fragments, antibodies differing in light chain composition, monocolonal and polyclonal antibodies of various classes, supercoiled DNA from linear duplexes, and single-stranded from double-stranded DNA.

CHT ceramic hydroxyapatite is available in two distinct material types, Type I and Type II (see table), and three particle sizes, 20, 40, and 80 µm (see figure). Both types retain elution characteristics similar to crystalline hydroxyapatite but also have unique properties of their own. CHT Type I has a higher protein binding capacity for acidic proteins than CHT Type II. CHT Type II has a lower protein binding capacity but gives better resolution for nucleic acids and certain proteins. Type II often provides superior selectivity and resolution for many species and classes of immunoglobulins, while having a very low affinity for albumin. The two types are often evaluated side by side to determine which material provides the maximum benefit in a given separation. Existing protocols that have been developed on crystalline hydroxyapatite can often be applied directly to CHT ceramic hydroxyapatite with little or no modification.

Mechanism of Action and Standard Chromatography

CHT ceramic hydroxyapatite 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 CHT ceramic hydroxyapatite via metal affinity. Repulsion effects and the geometric charge distribution on CHT ceramic hydroxyapatite provide unique selectivity. Typically, acidic, basic, and neutral proteins are bound to hydroxyapatite using a low ionic strength phosphate buffer. Elution is accomplished through the use of a sodium chloride or phosphate gradient of increasing strength. Regeneration of the support with phosphate buffers at neutral pH is followed by sanitization with up to 2 N NaOH. For more detailed information on process step development, refer to the CHT user's guide at **www.bio-rad.com/CHTGuide**.

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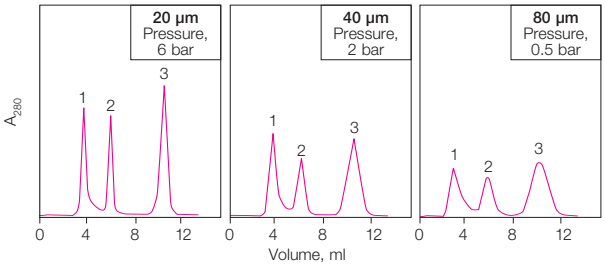
Specifications

Functional groups	Ca^{2+}, PO_4^{3-}, OH	
Particle sizes	20, 40, and 80 µm (nominal)	
Recommended linear flow rate	50–1,000 cm/hr	
Operating pH range	6.5–14	
Chemical compatibility (>24 hr)	1 N NaOH, 6 M urea, 8 M guanidine-HCl, ethanol, methanol, 100% acetonitrile	
Regeneration	0.4–0.5 M sodium phosphate, pH 7–7.5, is generally sufficient. If higher concentrations are needed, use potassium phosphate	
Sanitization	1–2 N NaOH	
Autoclavability (121ºC, 20 min)	Yes	
Packing density (g/ml packed bed)	0.63 g/ml	
	Type I	**Type II**
Dynamic binding capacity	>25 mg lysozyme/g	>12.5 mg lysozyme/g
Typical IgG binding capacities at 500 cm/hr	25–60 mg/ml	15–25 mg/ml
Nominal pore diameter	600–800 Å	800–1,000 Å
Maximum operating pressure	100 bar (1,500 psi)	100 bar (1,500 psi)

Note: A small amount (up to 5 mM) of sodium phosphate should be added to all unbuffered solutions as a counterion.



Effect of particle size on separation of proteins. A 10 µl sample of 10 mg/ml BSA (peak 1), 1.3 mg/ml lysozyme (peak 2), and 5 mg/ml cytochrome c (peak 3) was run on each 4 x 100 mm column packed with the indicated particle size of CHT ceramic hydroxyapatite at a flow rate of 478 cm/hr. The elution buffer was a linear gradient of 1–400 mM sodium phosphate, pH 6.8 over 15 min.

Storage

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

Technical Assistance

For more detailed information on process step development, use the recommended steps as described in the CHT Applications Guide (**www.bio-rad.com/CHTGuide**). All CHT ceramic hydroxyapatite supports have manufacturing processes registered with the United States Food and Drug Administration (FDA) by submission of a Type II Drug Master File (DMF). Regulatory support files are available upon request to companies entering into clinical trials. Bio-Rad Laboratories is an ISO 9001 registered corporation. For additional information and technical assistance, contact your local Bio-Rad office. In the U.S. and Canada, call 1-800-4BIORAD. 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
CHT Ceramic Hydroxyapatite, Type I	
158-2000	20 µm particle size, 10 g
157-0020	20 µm particle size, 100 g
157-0021	20 µm particle size, 1 kg
157-0025	20 µm particle size, 5 kg
158-4000	40 µm particle size, 10 g
157-0040	40 µm particle size, 100 g
157-0041	40 µm particle size, 1 kg
157-0045	40 µm particle size, 5 kg
158-8000	80 µm particle size, 10 g
157-0080	80 µm particle size, 100 g
157-0081	80 µm particle size, 1 kg
157-0085	80 µm particle size, 5 kg
732-4322	**Bio-Scale™ Mini CHT-I cartridge**, 40 µm, 1 x 5 ml
732-4324	**Bio-Scale Mini CHT-I cartridge**, 40 µm, 5 x 5 ml
CHT Ceramic Hydroxyapatite, Type II	
158-2200	20 µm particle size, 10 g
157-2000	20 µm particle size, 100 g
157-2100	20 µm particle size, 1 kg
157-2500	20 µm particle size, 5 kg
158-4200	40 µm particle size, 10 g
157-4000	40 µm particle size, 100 g
157-4100	40 µm particle size, 1 kg
157-4500	40 µm particle size, 5 kg
158-8200	80 µm particle size, 10 g
157-8000	80 µm particle size, 100 g
157-8100	80 µm particle size, 1 kg
157-8500	80 µm particle size, 5 kg
732-4332	**Bio-Scale Mini CHT-II cartridge**, 40 µm, 1 x 5 ml
732-4334	**Bio-Scale Mini CHT-II cartridge**, 40 µm, 5 x 5 ml
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.

Related Items

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.

For More Information

Request or download Bulletins 6086 and 6432.

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