

CHROMATOGRAPHY

CHT™ Ceramic Hydroxyapatite

- High throughput
- High physical and chemical stability
- Unparalleled selectivity
- Consistent reproducibility
- Three particle sizes for any application

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 ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) 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, monoclonal 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 than CHT Type II for acidic proteins. 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

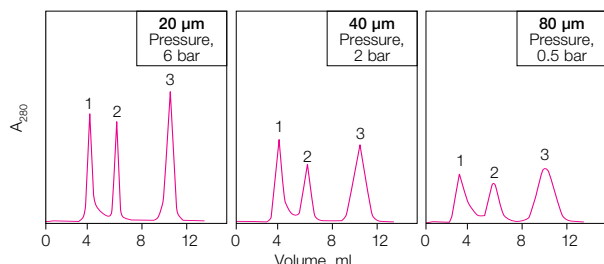
CHT ceramic hydroxyapatite interacts with biomolecules by multiple modes. Cation exchange occurs when negatively charged phosphate groups interact with protein amino groups. Much stronger coordination complexes can form between carboxyl clusters, phosphoryl moieties, or both, on biomolecules and the calcium sites on CHT ceramic hydroxyapatite via the mechanism of 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 M NaOH. For more detailed information, refer to the instruction manual.

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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 M NaOH, 6 M urea, 8 M guanidine-HCl, ethanol, methanol, 100% acetonitrile	
Regeneration	0.4–1.0 M phosphate buffer; 0.5 M sodium phosphate, pH 7; 1.0 M trisodium phosphate, pH 11–12	
Sanitization	1–2 M NaOH	
Autoclavability (121°C, 20 min)	Yes	
Packing density (g/ml packed bed)	0.63 g/ml	
Dynamic binding capacity	Type I >25 mg lysozyme/g	Type II >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 and Shelf Life

CHT ceramic hydroxyapatite should be stored in 0.1–1.0 M NaOH at room temperature. When sealed in the original container, unused CHT ceramic hydroxyapatite can be stored indefinitely in dry form at room temperature.

Technical Assistance

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 USA 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*	Catalog #	Description*
CHT Ceramic Hydroxyapatite, Type I		CHT Ceramic Hydroxyapatite, Type II	
158-2000	20 μm particle size, 10 g	158-2200	20 μm particle size, 10 g
157-0020	20 μm particle size, 100 g	157-2000	20 μm particle size, 100 g
157-0021	20 μm particle size, 1 kg	157-2100	20 μm particle size, 1 kg
157-0025	20 μm particle size, 5 kg	157-2500	20 μm particle size, 5 kg
158-4000	40 μm particle size, 10 g	158-4200	40 μm particle size, 10 g
157-0040	40 μm particle size, 100 g	157-4000	40 μm particle size, 100 g
157-0041	40 μm particle size, 1 kg	157-4100	40 μm particle size, 1 kg
157-0045	40 μm particle size, 5 kg	157-4500	40 μm particle size, 5 kg
158-8000	80 μm particle size, 10 g	158-8200	80 μm particle size, 10 g
157-0080	80 μm particle size, 100 g	157-8000	80 μm particle size, 100 g
157-0081	80 μm particle size, 1 kg	157-8100	80 μm particle size, 1 kg
157-0085	80 μm particle size, 5 kg	157-8500	80 μm particle size, 5 kg
732-4322	Bio-Scale™ Mini CHT-I cartridge, 40 μm, 1 x 5 ml	732-4332	Bio-Scale Mini CHT-II cartridge, 40 μm, 1 x 5 ml
732-4324	Bio-Scale Mini CHT-I cartridge, 40 μm, 5 x 5 ml	732-4334	Bio-Scale Mini CHT-II cartridge, 40 μm, 5 x 5 ml

* Larger quantities available on request.

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Germany 089 318 84 0 Greece 30 210 777 4396 Hong Kong 852 2789 3300 Hungary 36 1 455 8800 India 91 124 4029300 Israel 03 963 6050
Italy 39 02 216091 Japan 03 6361 7000 Korea 82 2 3473 4460 Mexico 52 555 488 7670 The Netherlands 0318 540666 New Zealand 0508 805 500
Norway 23 38 41 30 Poland 48 22 331 99 99 Portugal 351 21 472 7700 Russia 7 495 721 14 04 Singapore 65 6415 3188 South Africa 27 861 246 723
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