CHROMATOGRAPHY

CHT™ Ceramic Hydroxyapatite

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, 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.
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

**Recommended flow rate**: 50–1,000 cm/hr

**Typical Igg binding capacities at 500 cm/hr**
- 25–60 mg/ml
- 15–25 mg/ml

**Maximum operating pressure**: 100 bar (1,500 psi)

**Regeneration**: 0.4–1.0 M phosphate buffer; 0.5 M sodium phosphate, pH 7;
1.0 M trisodium phosphate, pH 11–12

**Chemical compatibility (>24 hr)**: 1 M NaOH, 6 M urea, 8 M guanidine-HCl, ethanol, methanol, 100% acetonitrile

**Operating pH range**: 6.5–14

**Particle sizes**: 20, 40, and 80 µm (nominal)

**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](http://www.bio-rad.com) for more information on Bio-Rad’s complete line of process chromatography supports.

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**Ordering Information**

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<th>Description*</th>
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* Larger quantities available on request.