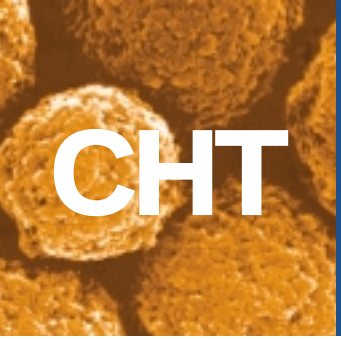


CHROMATOGRAPHY

# CHT™ Ceramic Hydroxyapatite

$Ca^{2+}$, $(PO_4)^{3-}$

## High-Resolution Protein Separations Using CHT Ceramic Hydroxyapatite

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


### Summary

Traditional crystalline hydroxyapatite is widely known for its unique separation properties and unparalleled selectivity. The fragile nature of the crystalline material has, however, limited its usefulness, particularly for large-scale processes. CHT ceramic hydroxyapatite overcomes the physical and chemical limitations of traditional crystalline hydroxyapatite and provides the throughput, stability, and reproducibility required for industrial biopharmaceutical manufacturing.

CHT ceramic hydroxyapatite is a chemically pure form of hydroxyapatite that has been sintered at high temperatures to yield a physically and chemically robust support. It possesses high protein binding capacities and can be used reproducibly for hundreds of cycles in large columns run at high flow rates. It 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 the separation properties of crystalline hydroxyapatite as well as possessing some unique properties of their own. Type I CHT has a higher protein binding capacity than Type II, and in particular, a higher capacity for acidic proteins. Type II often provides superior selectivity and resolution in the separation of many proteins, particularly various species, subspecies, and classes of antibodies, as well as nucleic acids. 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 the ceramic material with little or no modification.


**Table. Properties of CHT ceramic hydroxyapatite.**

| Characteristic | Type I | Type II |
|---|---|---|
| Functional groups | $Ca^{2+}$, $(PO_4)^{3-}$ | $Ca^{2+}$, $(PO_4)^{3-}$ |
| Dynamic binding capacity | >25 mg lysozyme per gram CHT | >12.5 mg lysozyme per gram CHT |
| Typical IgG binding capacities at 500 cm/hr | 10–50 mg/ml | 5–25 mg/ml |
| Particle sizes available | 20, 40, and 80 µm (nominal) | 20, 40, and 80 µm (nominal) |
| Nominal pore diameter | 600–800 Å | 800–1,000 Å |
| Maximum operating pressure | 60 bar (900 psi) | 100 bar (1,500 psi) |
| Recommended operating flow rates | 10–1,500 cm/hr | 10–1,500 cm/hr |
| pH stability | 5.5–14 | 5.5–14 |
| Regeneration | in 0.4–1.0 M phosphate buffer | in 0.4–1.0 M phosphate buffer |
| Sanitization | in 1–2 N NaOH | in 1–2 N NaOH |
| Autoclavability | 20 min at 121°C | 20 min at 121°C |
| **Chemical Compatability** | | |
| 1 N NaOH | >24 hr | >24 hr |
| 8 M urea | >24 hr | >24 hr |
| 8 M guanidine-HCl | >24 hr | >24 hr |
| Ethanol or methanol | >24 hr | >24 hr |
| 100% acetonitrile | >24 hr | >24 hr |

Note: A small amount of an appropriate counterion such as 0.1–10 mM $NaPO_4^-$ should be added to all unbuffered solutions.

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## Mechanism of Action and Standard Chromatography

CHT ceramic hydroxyapatite interacts with biomolecules by multiple modes. Electrostatic interactions occur between positively charged calcium ions and negatively charged phosphate groups on the support and charged moieties on the biomolecule's surface. Much stronger coordination complexes can form between clusters of carboxyl groups (on proteins) and calcium sites on CHT. Repulsion effects and the geometric charge distribution on CHT 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 phosphate gradient of increasing strength. Regeneration of the support with high-strength phosphate buffer is followed by sanitization with base. For more detailed information, refer to the instruction manual.

## Storage and Shelf Life

CHT ceramic hydroxyapatite should be stored in 20% ethanol v/v containing a small amount of an appropriate counterion such as 0.1–10 mM $NaPO_4^-$. Storage in phosphate buffer containing a bacteriostatic agent such as sodium azide ($NaN_3$) is also possible. Ceramic hydroxyapatite can be stored indefinitely in dry form at room temperature when sealed in the original container.

## Technical Assistance

All of the 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 and other products for life science research and production.

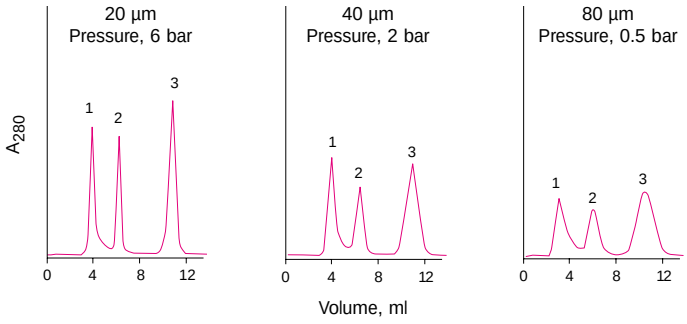


Figure. Effect of particle size on separation of proteins. A 10 µl sample of 10 mg/ml BSA (peak 1), 1.3 mg/ml lysozyme (peak 2), and 5 mg/ml cytochrome c (peak 3) was run on each 4 x 100 mm column packed with the indicated particle size at a flow rate of 478 cm/hr. The elution buffer was a linear gradient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

## Ordering Information

| Catalog # | Description |
|---|---|
| 158-2000 | CHT Ceramic Hydroxyapatite, Type I, 20 µm, 10 g |
| 157-0020 | CHT Ceramic Hydroxyapatite, Type I, 20 µm, 100 g |
| 157-0021 | CHT Ceramic Hydroxyapatite, Type I, 20 µm, 1 kg |
| 157-0025 | CHT Ceramic Hydroxyapatite, Type I, 20 µm, 5 kg |
| 158-4000 | CHT Ceramic Hydroxyapatite, Type I, 40 µm, 10 g |
| 157-0040 | CHT Ceramic Hydroxyapatite, Type I, 40 µm, 100 g |
| 157-0041 | CHT Ceramic Hydroxyapatite, Type I, 40 µm, 1 kg |
| 157-0045 | CHT Ceramic Hydroxyapatite, Type I, 40 µm, 5 kg |
| 158-8000 | CHT Ceramic Hydroxyapatite, Type I, 80 µm, 10 g |
| 157-0080 | CHT Ceramic Hydroxyapatite, Type I, 80 µm, 100 g |
| 157-0081 | CHT Ceramic Hydroxyapatite, Type I, 80 µm, 1 kg |
| 157-0085 | CHT Ceramic Hydroxyapatite, Type I, 80 µm, 5 kg |
| 158-2200 | CHT Ceramic Hydroxyapatite, Type II, 20 µm, 10 g |
| 157-2000 | CHT Ceramic Hydroxyapatite, Type II, 20 µm, 100 g |
| 157-2100 | CHT Ceramic Hydroxyapatite, Type II, 20 µm, 1 kg |
| 157-2500 | CHT Ceramic Hydroxyapatite, Type II, 20 µm, 5 kg |
| 158-4200 | CHT Ceramic Hydroxyapatite, Type II, 40 µm, 10 g |
| 157-4000 | CHT Ceramic Hydroxyapatite, Type II, 40 µm, 100 g |
| 157-4100 | CHT Ceramic Hydroxyapatite, Type II, 40 µm, 1 kg |
| 157-4500 | CHT Ceramic Hydroxyapatite, Type II, 40 µm, 5 kg |
| 158-8200 | CHT Ceramic Hydroxyapatite, Type II, 80 µm, 10 g |
| 157-8000 | CHT Ceramic Hydroxyapatite, Type II, 80 µm, 100 g |
| 157-8100 | CHT Ceramic Hydroxyapatite, Type II, 80 µm, 1 kg |
| 157-8500 | CHT Ceramic Hydroxyapatite, Type II, 80 µm, 5 kg |

Larger quantities available on request.


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