CHROMATOGRAPHY

CFT™ Ceramic Fluoroapatite

- Acidic protein separation for applications requiring pH as low as 5.0
- High-density particles for rapid, simple column packing
- Sintered at 400–700°C for a heavy-duty durable support
- Rigid particles for fast cleaning and equilibration
- Inorganic calcium phosphate backbone for distinct selectivities

A Robust Chromatography Support for Protein Purifications Requiring Acidic Conditions

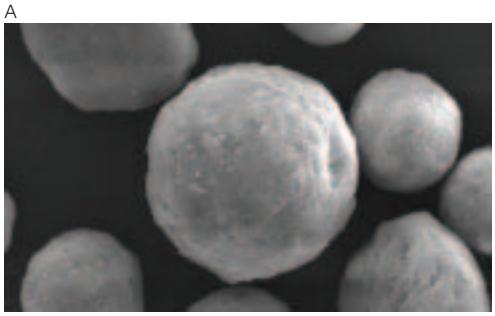
The newest addition to Bio-Rad's line of inorganic calcium phosphates is CFT ceramic fluoroapatite, a robust ceramic apatite able to sustain the rigors of protein separations requiring buffered conditions as low as pH 5. CFT ceramic fluoroapatite has separation characteristics similar to those of CHT™ ceramic hydroxyapatite, but now gives chromatographers the choice of purification conditions over a wider pH range. CFT may be used under acidic chromatography conditions to separate proteins with minimal effect on the solubility or lifetime of the support (Table 1). Its high tensile strength, chemical durability, and density enable it to provide the throughput and reproducibility required for biopharmaceutical manufacturing.

CFT is a rigid spherical macroporous support used in the purification of biologically active compounds (Figure 1). CFT is a composite of fluoroapatite and hydroxyapatite prepared by chemically converting hydroxyapatite nanocrystals to fluoroapatite with a fluorine reagent. This conversion goes to approximately 90% completion.

CFT is available in two distinct material types, Type I and Type II (Table 2), and one particle size, 40 µm. The two types are chemically identical, but have been sintered at different times and temperatures, resulting in a physically and chemically stable support.

CFT Type I and II behave similarly to their CHT counterparts, except when binding IgG. In the case of CFT, Type II has a higher dynamic binding capacity for IgG than Type I (Figure 2A); both types offer high binding capacities over a range of flow rates (Figure 2B). In general, protein selectivity is expected to be unaffected regardless of whether CFT or CHT is used. Solubility or apatite lifetime, however, will be less of a concern with CFT when lower pH buffer systems are required. To determine which material type provides optimal chromatographic performance, it is best to evaluate all four media (CFT and CHT, Types I and II).

A



B

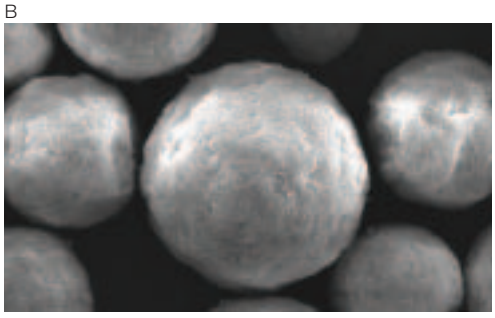


Fig. 1. Scanning electron micrograph showing the structure of CFT ceramic fluoroapatite beads, Types I (A) and II (B), at 1,000x magnification.

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Table 1. Chemical compatibilities.*

Solution	Compatibility	Storage Time
6 M guanidine-HCl	Yes	>24 hr
8 M urea	Yes	>24 hr
2 N NaOH	Yes	>24 hr
100% ethanol or methanol	Yes	>24 hr
1% SDS	Yes	>24 hr
Chelating agents	No	>24 hr
Chelating buffers (e.g., PIPES)	No	>24 hr
Organic acids or salts (e.g., malate, citrate, acetate)	With caution	>24 hr
Tris-HCl,** pH 7–9	Yes	>14 days
MES,*** pH 5–6.7	Yes	>14 days
NaOAc,*** pH 4.2–5.2	Yes	>14 days

* Solubility tests were carried out to determine whether CFT could be stored in common starting buffers that are known to affect the solubility of CHT. Calcium content was determined by inductively coupled plasma mass spectrometry on CFT stored at 37°C for 14 days or 24 hr in 200 ml of the indicated solutions. Calcium contents for treatments with Tris-HCl (pH 7.17), MES (pH 5.66), and NaOAc (pH 5.72) were 2.4, 24.2, and 23.1 ppm, respectively.

** Not known to dissolve CHT ceramic hydroxyapatite.

*** Known to dissolve CHT ceramic hydroxyapatite.

Mechanism of Action

CFT has unique selectivity for biomolecules, with several modes of interaction. Amino groups in proteins are attracted to phosphate sites (P-sites) in the apatite, but repelled by calcium sites (C-sites). The situation is reversed for carboxyl groups. Although amine binding to P-sites and the initial attraction of carboxyls to C-sites are electrostatic, the actual binding of carboxyls to C-sites involves formation of much stronger coordination complexes between C-sites and clusters of protein carboxyls. This was experimentally observed by evaluating the retention of proteins with carboxyl-to-sulfo group substitutions; binding is reduced dramatically even though net charge remains the same (Gorbunoff 1984b). Further evidence that carboxyl to C-site binding does not reflect a classical anion exchange interaction is that binding capacity diminishes for acidic proteins with increasing pH (Bernardi and Kawasaki 1968, Gorbunoff 1984a and 1984b, Ogawa and Hiraide 1996).

Table 2. Properties of CFT ceramic fluoroapatite.

Characteristic	Specification
Functional groups	Ca^{2+}, $(PO_4)^{3-}$, F^-
Dynamic binding capacities*	
Bovine IgG (Type I)	14–17 mg/ml
Bovine IgG (Type II)	30–32 mg/ml
Lysozyme (Type I)	25–30 mg/ml
Lysozyme (Type II)	17–21 mg/ml
Surface area	
Type I	33–36 m^2/g
Type II	16–18 m^2/g
Nominal pore diameter	600–800 Å
Maximum operating pressure	800 psi (55 bar or 5.5 MPa)
Nominal mean particle size	40 ± 4 µm
Nominal density	0.79–0.85 g/ml
Recommended linear flow rate	50–300 cm/hr
Operating pH	5–14
Storage pH range	11–14
Regeneration	3–5 column volumes (CV) of the following after every run:
Normal conditions	400 mM sodium phosphate, pH 6.8
Difficult conditions	400 mM trisodium phosphate, pH 11–12
Basic proteins (pI >7)	1–2 M NaCl or KCl, pH 10–12
Cleaning-in-place	Same as regeneration, or any of the following:
Lipid removal	1 CV H_2O, then 3–5 CV 70–100% methanol, ethanol, isopropyl alcohol, or acetonitrile, then 1 CV H_2O
	1% SDS
Other	6 M urea or 6 M guanidine-HCl
Sanitization	Perform cleaning-in-place, then 3–5 CV 1–2 N NaOH or KOH; exposure time ≥1 hr
Recommended column storage	0.1–1 N NaOH or KOH
Shelf life (dry, unused material)	>2 years

* Dynamic binding capacity conditions:

Samples	1 mg/ml bovine IgG or 2 mg/ml lysozyme in binding buffer ($QB_{1\%}$ determination)
Column volume	5.0 ml
Flow rate	Determined on a 1.1 x 5.2 cm column and run at 150 cm/hr
Binding buffer	10 mM sodium phosphate, pH 6.8
Elution buffer	400 mM sodium phosphate, pH 6.8

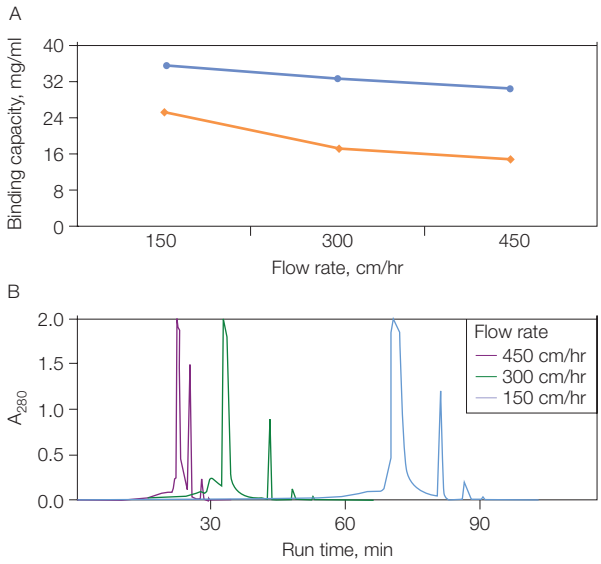

Fig. 2. Effect of flow rate on IgG capture. A, binding capacity of CFT Type I (orange) and Type II (blue). B, effect of flow rate on IgG capture using CFT Type II. Bovine IgG (1 mg/ml) was run on a 1.1 x 4.76 cm column packed with 4 g CFT until an A_{280} of 0.80 was reached. The loading buffer was 10 mM sodium phosphate, pH 6.75. Elution was performed using a step gradient from 10 to 400 mM sodium phosphate. The binding capacities were 37.0, 31.2, and 31.0 mg/ml, respectively, at 150, 300, and 450 cm/hr. Chromatography was performed on the BioLogic DuoFlow™ system.

Phosphate groups on proteins and other solutes interact more strongly with C-sites than do carboxyls (Kawasaki 1991). This is reflected in extremely strong binding by phosphoproteins (Bernardi and Cook 1960). Endotoxins also bind through the numerous phosphate groups on their core polysaccharide and lipid-A moieties (Homma et al. 1984).

Interestingly, DNA does not bind as strongly as expected for a phosphate-rich solute. The spacing of the phosphate groups along the backbone apparently prevents an ideal match with the steric distribution of C-sites (Bernardi 1971, Bernardi et al. 1972, Kawasaki 1991, Martinson 1973, Martinson and Wagenaar 1974). However, DNA/matrix interactions strengthen for longer nucleotides.

Standard Chromatography

Standard chromatography is performed using low-ionic-strength phosphate buffers (potassium or sodium) to bind acidic, basic, and neutral proteins. A gradient of increasing phosphate concentration is used to elute bound compounds. CFT should be regenerated after each run with high-strength phosphate buffer. Other effective cleaning-in-place agents include 1–2 M potassium or sodium chloride, 400 mM trisodium phosphate, 6 M urea or guanidine-HCl, and pure organic solvents. CFT should always be sanitized prior to storage with 1–2 N potassium or sodium hydroxide. For more detailed information on the handling and use of CFT, refer to the instruction manual.

Storage and Shelf Life

Recommended storage conditions for CFT ceramic fluoroapatite are 0.1–1.0 N potassium or sodium hydroxide at room temperature. Low concentrations of sodium or potassium phosphate buffer (pH 6.8) with 20% (v/v) ethanol or methanol may also be used. When sealed in its original container, ceramic fluoroapatite may be stored dry at room temperature for up to 2 years.

Technical Assistance

Bio-Rad Laboratories is an ISO 9001:2000 registered corporation. A regulatory support file on CFT is available upon request for companies entering into clinical trials. For additional information and technical assistance, contact your local Bio-Rad office. (In the USA and Canada, call 1-800-4BIORAD.)

Availability

CFT is available in both lab and process-scale sizes, ranging from 10 g to 5 kg amounts. Chromatographic performance of CFT Types I and II may be easily evaluated by requesting the lab sizes. In this case, the 10 g amount is recommended for initial testing of the media. For even greater convenience, prepacked cartridges containing the CFT media will also be made available.

Visit us on the Web at **www.bio-rad.com** for more information on Bio-Rad's complete line of chromatography media and other products for life science research and production.

References

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Ogawa T and Hiraide T, Effect of pH on gradient elution of different proteins on two types of ceramic hydroxyapatite, Am Lab 28, 17I–17L (1996)

Ordering Information

Catalog #	Description
CFT Ceramic Fluoroapatite, Types I and II	
158-5000	CFT Ceramic Fluoroapatite, Type I, 40 µm, 10 g
158-5200	CFT Ceramic Fluoroapatite, Type II, 40 µm, 10 g
157-0050	CFT Ceramic Fluoroapatite, Type I, 40 µm, 100 g
157-5000	CFT Ceramic Fluoroapatite, Type II, 40 µm, 100 g
157-0051	CFT Ceramic Fluoroapatite, Type I, 40 µm, 1 kg
157-5100	CFT Ceramic Fluoroapatite, Type II, 40 µm, 1 kg
157-0055	CFT Ceramic Fluoroapatite, Type I, 40 µm, 5 kg
157-5500	CFT Ceramic Fluoroapatite, Type II, 40 µm, 5 kg

Related Products

Catalog #	Description
CHT Ceramic Hydroxyapatite, Type I	
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
CHT Ceramic Hydroxyapatite, Type II	
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
Prepacked Econo-Pac® Cartridges	
732-0081	CHT Ceramic Hydroxyapatite, Type II Support, 1 x 5 ml
732-0085	CHT Ceramic Hydroxyapatite, Type II Support, 5 x 5 ml
732-0083	CHT Ceramic Hydroxyapatite, Type II Support, 5 x 1 ml

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Bulletin 3111 US/EG Rev A *04-0241 0704 Sig 1103*