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# CFT™ Ceramic Fluoroapatite

## Instruction Manual

Please read these instructions prior to using CFT ceramic fluoroapatite. If you have any questions or comments regarding these instructions, please contact your local Bio-Rad representative.

**BIO-RAD**

# Table of Contents

<b>Section 1. Product Description</b> .....	<b>1</b>
1.1 Characteristics of CFT Ceramic Fluoroapatite.....	2
1.2 Chemical Compatibility.....	3
1.3 General Handling .....	3
<b>Section 2. Column Packing</b> .....	<b>4</b>
2.1 Recommended Column Packing Buffer System.....	4
2.2 Small Column Packing — Slurry Packing .....	4
2.3 CFT pH Change and Equilibration .....	5
2.4 Column Qualification .....	5
<b>Section 3. Chromatography</b> .....	<b>5</b>
<b>Section 4. Cleaning-in-Place and Regeneration</b> .....	<b>6</b>
<b>Section 5. Sanitization</b> .....	<b>6</b>
<b>Section 6. Storage</b> .....	<b>7</b>
<b>Section 7. Tips and General Information</b> .....	<b>7</b>
<b>Section 8. Reference</b> .....	<b>8</b>
<b>Section 9. Ordering Information</b> .....	<b>9</b>

# Section 1. Product Description

CFT ceramic fluoroapatite ( $\text{Ca}_5(\text{PO}_4)_3\text{F}_2$ ) is an inorganic calcium phosphate used in the chromatographic separation of biomolecules. CFT has separation characteristics similar to CHT™ ceramic hydroxyapatite, but can be used under chromatographic conditions as low as pH 5 to separate acidic proteins.

CFT is a rigid spherical macroporous chromatographic support that has been sintered at high temperatures to modify its microcrystalline lattice into a fused ceramic. The sintering process improves the mechanical strength of the particle. It is a composite of fluoroapatite and hydroxyapatite prepared by chemically converting hydroxyapatite nanocrystals with a fluorine reagent. The molecular weight of CFT is 1,008.6. The solubility constant is approximately  $1.0 \times 10^{-60}$ . CFT is available in two distinct material types, Type I, sintered at 400°C, and Type II, sintered at 700°C, 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 in the case of IgG. CFT Type II has a higher dynamic binding capacity for IgG than CFT Type I, whereas CHT Type I has a higher dynamic binding capacity for IgG than CHT Type II. Otherwise, the protein selectivity of these two products is comparable. Solubility or apatite lifetime will be less of a concern with CFT when lower pH buffer systems are required. In order to determine which material types provide optimal chromatographic performance, it is beneficial to evaluate both CFT and CHT, and both Types I and II.

## 1.1 Characteristics of CFT Ceramic Fluoroapatite

<b>Characteristics</b>	<b>Types I and II</b>
Functional groups	Ca <sup>2+</sup> , PO <sub>4</sub> <sup>3-</sup> , F <sup>-</sup>
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 <sup>2</sup> /g
Type II	16–18m <sup>2</sup> /g
Maximum operating pressure	800 psi or 55 bar or 5.5 MPa
Nominal mean particle size (µm)	40 ± 4
Nominal density	0.79–0.85 g/ml
Recommended linear flow rate	50–300 cm/hr
Operating pH range	5–14
Storage pH range	11–14
Regeneration	3–5 column volumes (CV) of the following after every run:
Normal	400 mM sodium phosphate, pH 6.8
Difficult	400 mM trisodium phosphate, pH 11–12
Basic proteins (pI > 7)	1–2 N NaCl or KCl, pH 10–12
Cleaning-in-place (CIP)	Same as regeneration and/or any of the following:
Lipids	1 CV H <sub>2</sub> O, then 3–5 CV 70–100% methanol, ethanol, isopropanol, or acetonitrile, then 1 CV H <sub>2</sub> O
	1% SDS
Other	6 M urea or 6 M guanidine-HCl
Sanitization	Perform a CIP, 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 <sub>1%</sub> 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

## 1.2 Chemical Compatibility

Solution	Compatibility	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/salts (e.g., malate, citrate, acetate)	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

\* Not known to dissolve CHT ceramic hydroxyapatite

\*\*Known to dissolve CHT ceramic hydroxyapatite

Solubility tests were carried out to determine if 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 (ICP-MS) on CFT stored at 37°C for 14 days in the indicated buffers.

## 1.3 General Handling

CFT is a rigid support that can operate under high flow rates and pressures.

Particles can be fractured through mechanical stress and handling, e.g.:

- Stirring with magnetic stirrers
- Operating at too high a speed or for too long with axial or radial stirrers
- Recycling slurries with peristaltic or diaphragm pumps

If fines are created, they can cause an increase in the column backpressure and may reduce binding capacity.

# Section 2. Column Packing

## 2.1 Recommended Column Packing Buffer System

A solution of 200 mM dibasic sodium phosphate, pH 9–10, is recommended for packing CFT ceramic fluoroapatite. Historically, solutions up to 400 mM sodium phosphate have been used, but there is no added benefit from using these higher concentrations.

## 2.2 Small Column Packing — Slurry Packing

Slurry packing is preferred for small columns. Use this method for packing 40  $\mu\text{m}$  CFT ceramic fluoroapatite into 5–50 mm ID columns. For best results, a bed height of 5–30 cm should be used.

1. Use a column that is 2.5 times longer than the required bed height. Close the outlet valve.
2. Make a 50% (v/v) slurry of CFT in degassed 200 mM phosphate buffer, pH 9–10. Suspend the CFT by gently swirling or stirring with a plastic rod. **Do not use a magnetic stirrer.**
3. Fill the column to about 10% of its volume with buffer. Ensure that the bottom bed support is fully saturated and free of bubbles.
4. Pour the slurry into the column and insert the top flow adaptor. Make sure the tubing is open to allow buffer to flow out through the top adaptor along with any air as the adaptor is inserted into the column tube.
5. Secure the top adaptor and attach the buffer line from the pump.
6. Open the column outlet and pump buffer through the column at ~400–600 cm/hr for 5–10 min or at the maximum pressure allowed by the column hardware.
7. Turn off the pump and close the column outlet. Open the tubing of the top flow adaptor again to allow liquid to exit the column as the flow adaptor is lowered to rest firmly against the top of the CFT bed.
8. Repeat cycles of flow followed by adjustment of the flow adaptor until the CFT bed height stops decreasing with flow.
9. Equilibrate.

## 2.3 CFT pH Change and Equilibration

The most rapid method for changing the pH of CFT is with 2.5–5 CV of >200 mM sodium phosphate at the desired pH.

If reequilibrating from the sanitizing-in-place (SIP) solution or storage in strong base, use 400 mM sodium phosphate at the desired pH. Once the desired pH is achieved, wash with equilibration buffer, e.g., 10 mM sodium phosphate, at the same desired pH.

## 2.4 Column Qualification

The packing efficiency of CFT can be evaluated by calculating HETP (height equivalent to theoretical plate) and peak symmetry. Use 2–5% (v/v) acetone, 0.1% (w/v) DL-tryptophan, or salt. Due to the dynamics of counterion exchange, a concentration of at least 0.15 M NaCl should be included in the equilibration buffer if salt is being used. For calculation of HETP, up to 1.75 M NaCl may be used.

# Section 3. Chromatography

CFT chromatography is performed similarly to CHT chromatography; phosphate buffer systems with increasing concentrations of phosphate elute bound molecules. The following is the recommended protocol for a typical separation:

1. Samples containing high concentrations of phosphates should be diluted to the concentration of the starting buffer (typically 10 mM phosphate, pH 6.8).
2. Buffer solutions and samples should be filtered through a 0.2–0.45  $\mu\text{m}$  filter before use.
3. Apply the sample and allow unbound material to pass through the column.
4. Elute with a gradient of increasing concentration of phosphate buffer (10–400 mM). If performing chromatography at  $\leq 12^\circ\text{C}$ , use potassium phosphate because sodium phosphate exhibits lowered solubility at these lower temperatures.

## Section 4. Cleaning-in-Place and Regeneration

CFT should be regenerated at the completion of each run with 3–5 CV of 400–500 mM potassium or sodium phosphate buffer (pH 6.8). Remove more tightly bound and basic proteins ( $pI > 7$ ) with 3–5 CV of 1–2 M KCl or NaCl, or 400 mM trisodium phosphate (pH 11–12). Rinse the column with 1 CV of H<sub>2</sub>O between any change of salt to avoid precipitation.

Urea (8 M) and guanidine-HCl (6 M) are effective cleaning agents (see Chemical Compatibility above).

Pure organic solvents or different percentage alcohol solutions in water may be used to clean CFT. Since the viscosity of alcohol-water solutions is greater than that of water, lower the flow rate when changing from aqueous to organic solvent solutions. Before cleaning with 70–100% ethanol, methanol, isopropyl alcohol, or acetonitrile, rinse the column with 1 CV of distilled water to avoid salt precipitation. After cleaning with the organic solvent, rinse with 1 CV of water followed by 1 CV of 5 mM sodium or potassium phosphate buffer (pH 6.8).

## Section 5. Sanitization

CFT should always be sanitized prior to storage to avoid growth of microorganisms. Such contamination can lead to localized production of acid, resulting in damage to the support. CFT is stable in 2 M potassium or sodium hydroxide. To sanitize, first regenerate the column with 400–500 mM phosphate buffer as mentioned above. Then rinse the column with 3–5 CV (1 hr exposure) of 1–2 M potassium or sodium hydroxide.

When reequilibrating from strong hydroxide solutions, rinse with 400–500 mM potassium or sodium phosphate buffer at the desired pH until the pH of the eluent is equal to the pH of the applied buffer (approximately 3–5 CV).



## Section 6. Storage

Recommended storage of CFT is in 0.1–1 M sodium or potassium hydroxide at room temperature. It can also be stored in low concentrations of sodium or potassium phosphate buffer (pH 6.8) with 20% (v/v) ethanol or methanol.

Unused CFT should be stored in its original sealed container at room temperature.

## Section 7. Tips and General Information

1. **Elution buffers** — For most applications, elute bound fractions with linear or step gradients of potassium or sodium phosphate at neutral pH (6.8–7.2), going from low (5–10 mM) to high (400 mM) phosphate buffer concentrations. Use potassium phosphate when operating at temperatures below 12°C because sodium phosphate can precipitate at the lower temperatures.
2. **Method optimization** — Binding capacity and retention time decrease with increasing pH. CFT ceramic fluoroapatite is a calcium phosphate compound that dissolves at low pH. When used at pH 4, the CFT degrades quickly. After 100 cycles in pH 5 phosphate buffer, there is a small loss in mass of CFT. After 100 cycles in pH 5.5 phosphate buffer, there is no noticeable loss in mass. Loading can be performed in low concentrations of phosphate (10–20 mM) between pH 5.5 and 6.8, and elution can be done with linear or step gradients at higher phosphate concentrations (up to ~400 mM).
3. **Other buffers** — Special attention to selection of the buffer system is recommended when deviating from standard phosphate buffer because the stability of CFT may be adversely affected. Chelating buffers, such as PIPES, should not be used with CFT.
4. **Chelating agents** — Do not use EDTA or other chelating agents in the sample or the buffers. Chelating agents bind to the calcium in the CFT and eventually dissolve the support.
5. **Sodium chloride** — Basic proteins ( $pI > 7$ ) elute with NaCl, whereas acidic proteins ( $pI < 7$ ) do not.
6. **Calcium carbonate** — Carbonate-free water should be used to prepare all solutions. Carbonate reacts with calcium ions to form a crust of calcium carbonate at the top of the column. The severity or rate of carbonate buildup on CFT is dependent on the quality and volume of water used.

7. **Rigidity** — CFT is very rigid and can operate at high flow rates and operating pressures. However, because it is a ceramic, it is susceptible to mechanical damage. While making slurries, be careful to minimize the generation of fines. The use of mechanical stirrers at high rpm and stir bars should be avoided.

## Section 8. Reference

Sato T et al., Ceramic fluoroapatite beads for chromatography of proteins and amino acids, *Chromatography* 19, 191–199 (1998)

# Section 9. Ordering Information

## **Catalog #      Description**

### **CFT Ceramic Fluoroapatite**

158-5000	CFT Ceramic Fluoroapatite, Type I, 40 $\mu$ m, 10 g
158-5200	CFT Ceramic Fluoroapatite, Type II, 40 $\mu$ m, 10 g
157-0050	CFT Ceramic Fluoroapatite, Type I, 40 $\mu$ m, 100 g
157-5000	CFT Ceramic Fluoroapatite, Type II, 40 $\mu$ m, 100 g
157-0051	CFT Ceramic Fluoroapatite, Type I, 40 $\mu$ m, 1 kg
157-5100	CFT Ceramic Fluoroapatite, Type II, 40 $\mu$ m, 1 kg
157-0055	CFT Ceramic Fluoroapatite, Type I, 40 $\mu$ m, 5 kg
157-5500	CFT Ceramic Fluoroapatite, Type II, 40 $\mu$ m, 5 kg

### **CHT Ceramic Hydroxyapatite**

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

**Bio-Rad Laboratories, Inc.**

2000 Alfred Nobel Drive, Hercules, CA 94547 USA

510-741-1000

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