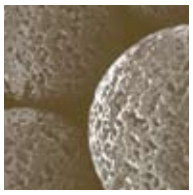

CHROMATOGRAPHY

CFT™ Ceramic Fluoroapatite Media

- Acidic protein separation for applications requiring pH as low as 5.0
- Sintered at high temperature for a heavy-duty durable support
- Rigid particles for fast cleaning and equilibration
- Inorganic calcium phosphate backbone for distinct selectivities

Obtain High Selectivity Under Demanding Low pH Conditions

CFT ceramic fluoroapatite is a rigid, spherical, macroporous support used in the purification of biologically significant compounds. CFT is a composite of fluoroapatite and hydroxyapatite prepared by chemically converting hydroxyapatite nanocrystals to fluoroapatite with a fluorine reagent. The chemical conversion allows CFT media to withstand the rigors of protein separations requiring acidic-buffered conditions (pH 5–6.5).

CFT ceramic fluoroapatite possesses separation characteristics similar to those of the more widely known CHT™ ceramic hydroxyapatite. However, when CFT is used, the process scientist can perform purification across a range of lower pH values to obtain optimal results for the targeted biomolecule. CFT can be used under stringent chromatography conditions to separate acidic proteins with minimal compromise to the solubility or lifespan of the apatite. Its tensile strength, chemical durability, and density enable it to provide the throughput and reproducibility required for process-scale manufacturing of biopharmaceuticals.

CFT Type II is available in a 40 µm particle size and is sintered at high temperatures to produce physically and chemically stable bioprocess media. CFT Type II maintains its highest IgG binding capacity (34–37 mg/ml) at pH 5.0–6.0 (Figure 1) and over a range of flow rates (Figure 2). When process requirements call for buffered conditions at low pH values, solubility and lifespan will be less of a concern with CFT media than with CHT ceramic hydroxyapatite (Figure 3). To determine which type of apatite provides optimal chromatographic performance, an evaluation of CFT and CHT media should be performed with attention to buffer pH and selectivity.

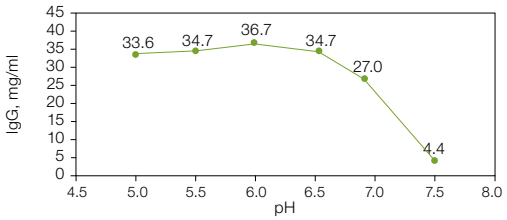

Fig 1. IgG binding vs. pH using CFT ceramic fluoroapatite media.

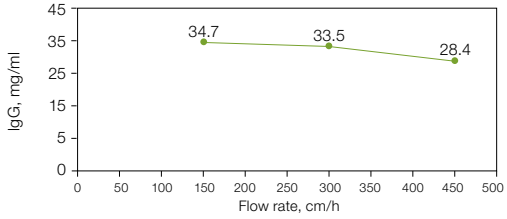

Fig 2. IgG binding vs. flow rate using CFT ceramic fluoroapatite media.

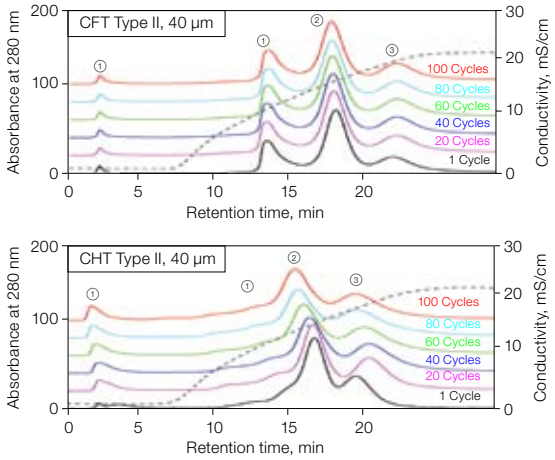

Fig 3. Separation of a mixture of three proteins demonstrated stability of CFT vs. CHT over 100 cycles at pH 5.0.

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Specifications

Functional groups	Ca^{2}+, $PO4^{3-}$, F–
Particle sizes	40 ± 4 µm
Recommended linear flow rate	300 cm/hr
Operating pH range	5–14
Chemical compatibility	2 M NaOH, 6 M guanidine-HCl, 8 M urea, 0.1 M sodium acetate, pH 5.7
Regeneration	
Normal conditions	400 mM sodium phosphate, pH 7.4
Difficult conditions	400–1,000 mM sodium phosphate, pH 11–12
Sanitization	1–2 M NaOH or KOH
Autoclavability (121°C, 20 min)	Yes
Packing density (g/ml packed bed)	0.86 g/ml
Dynamic binding capacity	14–21.5 mg lysozyme/g
Typical IgG binding capacities 300 cm/hr	33 mg/ml
Nominal pore diameter	600–800 Å
Maximum operating pressure	55 bar (800 psi)

Note: A small amount (up to 5 mM) of sodium phosphate should be added to all unbuffered solutions as a counterion.

Mechanism of Action and Standard Chromatography

The unique selectivity of CFT ceramic fluoroapatite is attributed to its multiple interactions with biomolecules. Amino groups are attracted to phosphate sites (P-sites) 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. Phosphoryl groups on proteins and other solutes interact even more strongly with C-sites than do carboxyl groups. The role of fluoride ions in the chromatographic interaction with biomolecules is unclear, but the partial substitution of hydroxyl groups by fluoride ions improves the stability of CFT ceramic fluoroapatite under increasingly acidic conditions.

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

Storage and Shelf Life

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

Technical Assistance

A regulatory support file on CFT is available upon request. Bio-Rad Laboratories Inc., 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.

Reference

Schubert S and Freitag R (2009). Investigation of the interaction mechanism of the recombinant human antibody MDJ8 and its fragments with chromatographic apatite phases. J Chromatogr A 1216, 3831–3840.

Schubert S and Freitag R (2007). Comparison of ceramic hydroxy- and fluoroapatite versus protein A/G–based resins in the isolation of a recombinant human antibody from cell culture supernatant. J Chromatogr A 1142, 106–113.

Ordering Information

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

Related Items

CHT Ceramic Hydroxyapatite, Type II, 20 µm	
158-2200	CHT Ceramic Hydroxyapatite, 10 g
157-2000	CHT Ceramic Hydroxyapatite, 100 g
157-2100	CHT Ceramic Hydroxyapatite, 1 kg
157-2500	CHT Ceramic Hydroxyapatite, 5 kg
CHT Ceramic Hydroxyapatite, Type II, 40 µm	
158-4200	CHT Ceramic Hydroxyapatite, 10 g
157-4000	CHT Ceramic Hydroxyapatite, 100 g
157-4100	CHT Ceramic Hydroxyapatite, 1 kg
157-4500	CHT Ceramic Hydroxyapatite, 5 kg
CHT Ceramic Hydroxyapatite, Type II, 80 µm	
158-8200	CHT Ceramic Hydroxyapatite, 10 g
157-8000	CHT Ceramic Hydroxyapatite, 100 g
157-8100	CHT Ceramic Hydroxyapatite, 1 kg
157-8500	CHT Ceramic Hydroxyapatite, 5 kg

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