CHROMATOGRAPHY

CHT Ceramic Hydroxyapatite XT Media

- High physical and chemical stability
- Unique selectivity
- Efficient single-step clearance of aggregates and other impurities
- Straightforward column packing
- Full regulatory support

Robust, Long-Lasting Media for Repeated Use and Exceptional Resolution

Introduction

CHT Ceramic Hydroxyapatite Media is a spherical, macroporous form of hydroxyapatite that has been sintered at high temperatures to yield a physically and chemically robust support. It is ideal for large-scale bioprocess applications, providing consistent results at scales of at least 1.8 meters. CHT Ceramic Hydroxyapatite provides unique separation properties along with unmatched selectivity and resolution. It can be used in applications such as purification of enzymes, monoclonal and polyclonal antibodies of various classes, antibody fragments, and bispecific antibodies; efficient isolation and purification of viruses and virus-like particles (VLPs); and separation of supercoiled DNA from linear duplexes as well as single stranded from double-stranded DNA.

High Stability and Unique Selectivity

CHT Ceramic Hydroxyapatite XT Media (CHT XT) is the latest addition to the existing line of ceramic hydroxyapatite chromatography media encompassing CHT Type I and Type II. The technical features of CHT XT are listed in Table 1. It is designed to provide added robustness and lifetime. The chemical composition of CHT XT is $(Ca_{10}(PO_4)_6(OH)_2)$ and it is a mixed-mode media. Biomolecules can interact with CHT through calcium affinity interactions and/or cation exchange interactions. Please refer to the CHT Applications Guide (bio-rad.com/CHTGuide) for details on both these interactions.

Table 1. Properties of CHT Ceramic Hydroxyapatite XT.

Property	Description
Function	Mixed-mode: cation exchange (phosphate) and affinity (calcium)
Functional group	Ca^{2+}, PO_4, OH
Median particle size	40 ± 4 µm
Dynamic binding capacity	17–25 mg Lysozyme/g* ≥ 60 mg/ml mAb G** (pI 9.2)
Recommended linear flow rate	50–1,000 cm/hr***
Maximum operating pressure	10 bar (150 psi)
Tap settled density	0.67**** g/ml
Compression factor	Incompressible
pH stability	6.5–14
Shipping form	Dry powder
Regeneration	0.4 to 0.5 M sodium phosphate, pH 7.0–7.5; 1 M trisodium phosphate, pH 11–12. If higher concentrations of sodium phosphate needed, use 0.4–1.0 M potassium phosphate
Sanitization	1–2 M sodium hydroxide
Autoclavability (bulk)	121°C, 20 min in phosphate buffered saline, pH 7
Storage conditions	0.1 M sodium hydroxide
Chemical compatibility	1 M sodium hydroxide, 8 M Urea, 6 M guanidine hydrochloride, ethanol, methanol, 100% acetonitrile, 0.4–1.0 M phosphate buffer, 0.5 M sodium phosphate, pH 7, 1.0 M trisodium phosphate, pH 11–12
Shelf life	5 years

* At 500 cm/hr with 10 mM sodium phosphate, pH 6.8.
** At 100 cm/hr with 5 mM sodium phosphate, 25 mM sodium chloride, pH 7 in a 0.5 x 9.5 cm column.
*** Tested on a 0.4 x 10 cm column packed at 150 cm/hr.
**** Value from preliminary data.
A small amount (up to 5 mM) of sodium phosphate should be added to all unbuffered solutions as a counter ion.

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Under appropriate buffer conditions, CHT XT can be used for a large number of purification cycles (Table 2). Chromatographically, CHT XT maintains the unique separation properties expected from CHT, including efficient resolution (Figure 1) and aggregate removal (Figure 2). Structurally, the three CHT Ceramic Hydroxyapatite types differ in their pore diameters and, therefore, in protein separation uses. This offers flexibility in selecting the CHT type that provides maximum benefit in a given separation. CHT Type I and XT have higher protein binding capacities than CHT Type II due to their large surface areas. CHT Type II, however, provides higher capacity for large molecules such as IgM and viruses as it has the largest pore diameter of the three CHT types.

Long Lifetime Ensures Repeated Reusability

CHT XT can be used for over 70 cycles under the conditions listed in Table 2, making it ideal for manufacturing processes.

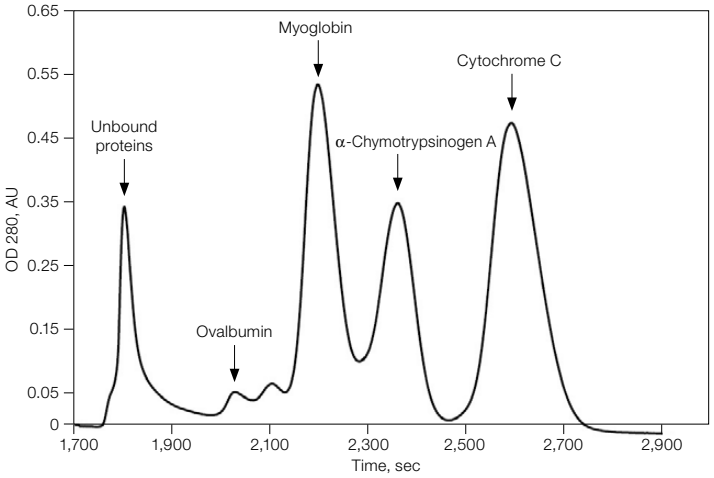
Table 2. Column lifecycle study of CHT XT. A 20 x 20 cm column was packed with CHT XT and cycled continuously at 140 cm/hr using buffer protocols I or II until a column backpressure of 3 bar was reached.

Protocol	Number of Cycles
Protocol I	139
Protocol II	74

Protocol I:	
Pre-equilibration:	0.4 M sodium phosphate, pH 7.0, 4 column volumes (CV)
Equilibration, Loading and wash:	5 mM sodium phosphate, 0.1 M sodium chloride, pH 6.5, 15 CV
Elution:	5 mM sodium phosphate, 0.55 M sodium chloride, pH 6.5, 4 CV
Stripping:	0.4 M sodium phosphate, pH 7, 3 CV
Sanitization:	1 M sodium hydroxide, 3 CV
Protocol II:	
Pre-equilibration:	0.4 M sodium phosphate, pH 6.5, 4 CV
Equilibration, Loading and wash:	5 mM sodium phosphate, pH 6.5, 15 CV
Elution:	5 mM sodium phosphate, 0.55 M sodium chloride, pH 6.5, 4 CV
Stripping:	0.4 M sodium phosphate, pH 6.5, 3 CV
Sanitization:	1 M sodium hydroxide, 1 M sodium chloride, 3 CV

Efficient Resolution of Proteins

A protein mixture was used to demonstrate the resolution properties of CHT XT. Figure 1 shows the efficient separation of the mixture, which consisted of four different proteins.



Flow rate:	1.57 ml/min
Pre-equilibrium:	400 mM sodium phosphate, pH 6.8, 4 CV
Loading buffer:	5 mM sodium phosphate, pH 6.8, 15 CV
Post-load wash:	5 mM sodium phosphate, pH 6.8, 1 CV
Elution:	Linear gradient elution 0–75% 400 mM sodium phosphate, pH 6.8, over 15 CV
Stripping:	400 mM sodium phosphate, pH 6.8, 3 CV
Sanitization:	1 M sodium hydroxide, 3 CV

Fig. 1. Separation of protein standards. A mixture of the following protein samples in 10 ml of 5 mM sodium phosphate, pH 6.8, was used for purification: 120 mg of ovalbumin, 75 mg of myoglobin, 60 mg of α-chymotrypsinogen A, 75 mg of cytochrome C. One hundred microliters of this mixture was loaded onto a 0.7 x 5.4 cm column with a packed bed volume of 2 ml of CHT XT.

Aggregate Removal during Monoclonal Antibody Purification

A monoclonal antibody sample (mAb G) was used to test the aggregate removal and antibody purification capability of CHT XT. The mAb G sample contained a significant amount (~28%) of aggregate as shown by size exclusion chromatography (SEC) analysis (Figure 2A). This sample was purified using CHT XT (Figure 2B) and the eluate from the resultant peak was again analyzed by SEC (Figure 2C). Complete separation of the mAb aggregates from the monomers was observed, demonstrating that CHT XT is highly effective at aggregate removal during monoclonal antibody purification.

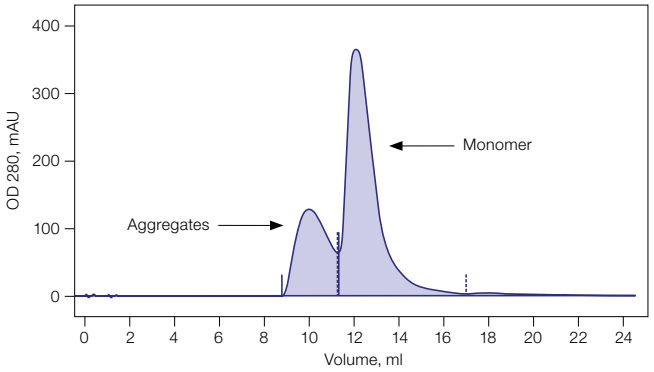
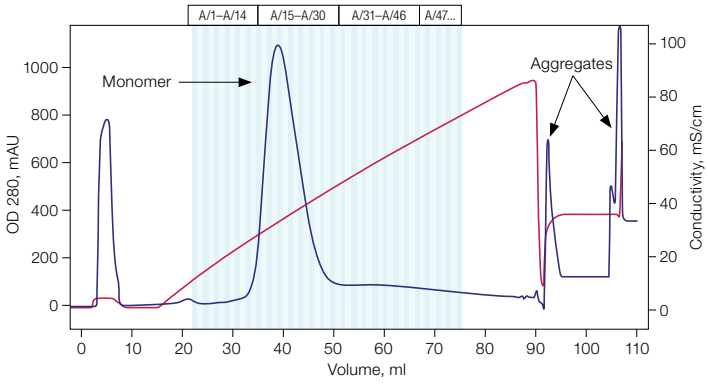


Fig. 2A. Size exclusion chromatography (SEC) of mAb G load. SEC showing the level of mAb G aggregates in the load. mAb G was loaded on a 10 x 300 mm Bio-Rad ENrich SEC 650 Column with a packed bed volume of 23.56 ml equilibrated in PBS. OD 280 (—).



Sample load:	40 mg mAb G sample with aggregates
Column:	1.85 ml CHT XT packed in a 0.5 x 9.4 cm column
Flow rate:	1 ml/min
Equilibration:	10 mM sodium phosphate, pH 6.5, 10 CV
Wash:	10 mM sodium phosphate, pH 6.5, 3 CV
Elution:	Linear gradient elution 0–100% 10 mM sodium phosphate, 1 M sodium chloride, pH 6.5
Fraction collection:	1 ml fraction size over 40 CV
Stripping:	0.4 M sodium phosphate, pH 7, 4 CV
Sanitization:	1 M sodium hydroxide, 3 CV

Fig. 2B. mAb G purification profile. Elution profile showing separation of the monomer from higher molecular weight impurities. OD 280 (—); conductivity (—).

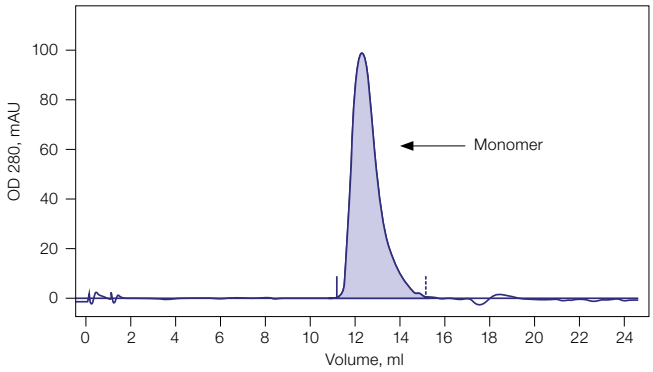


Fig. 2C. Final SEC profile of mAb G monomer pool. The SEC profile of the pooled fractions from CV 13-27 (A/13–A/27 in Figure 2B) confirms aggregate clearance from the mAb G monomer. OD 280 (—).

Storage

Columns packed with CHT XT can be stored in 0.1 M sodium hydroxide. In dry powder form, CHT XT should be stored in a closed container at room temperature. When sealed in the original container, unused CHT XT can be stored in the dry form for at least five years at room temperature.

Technical Assistance

A regulatory support file is available upon request.

Bio-Rad Laboratories Inc. is an ISO 13485 registered corporation. For detailed information on process development, refer to the CHT Applications Guide (bio-rad.com/CHTGuide).

For additional information and technical assistance, contact your local Bio-Rad office or email our process specialists at **process@bio-rad.com**. In the U.S. and Canada, call **1-800-4BIORAD**.

Screen this resin for your application by visiting bio-rad.com/web/ResinSample to request a sample.

Visit us on the web at bio-rad.com/ProcessResins for more information on Bio-Rad's complete line of process chromatography supports.

Ordering Information

Catalog #	Description
CHT Ceramic Hydroxyapatite XT Media	
12002457	**CHT Ceramic Hydroxyapatite XT**, 40 µm, 10 g
12002454	**CHT Ceramic Hydroxyapatite XT**, 40 µm, 100 g
12002456	**CHT Ceramic Hydroxyapatite XT**, 40 µm, 1 kg
12002455	**CHT Ceramic Hydroxyapatite XT**, 40 µm, 5 kg
Foresight Prepacked Columns	
12003150	**Foresight CHT XT Column**, 40 µm, 1 ml
12003149	**Foresight CHT XT Column**, 40 µm, 5 ml
Foresight Prepacked Chromatography Filter Plates*	
12003151	**Foresight CHT XT Plates**, 40 µm, 20 µl
Foresight RoboColumn Units**	
12003152	**Foresight CHT XT RoboColumn Units**, 40 µm, 200 µl
12003148	**Foresight CHT XT RoboColumn Units**, 40 µm, 600 µl

Related Items

Catalog #	Description
CHT Ceramic Hydroxyapatite Media, Type I	
1582000	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 10 g
1570020	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 100 g
157-0021	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 1 kg
157-0025	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 5 kg
1584000	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 10 g
1570040	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 100 g
157-0041	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 1 kg
157-0045	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 5 kg
1588000	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 10 g
1570080	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 100 g
157-0081	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 1 kg
157-0085	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 5 kg
CHT Ceramic Hydroxyapatite Media, Type II	
1582200	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 10 g
1572000	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 100 g
157-2100	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 1 kg
157-2500	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 5 kg
1584200	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 10 g
1574000	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 100 g
157-4100	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 1 kg
157-4500	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 5 kg
1588200	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 10 g
1578000	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 100 g
157-8100	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 1 kg
157-8500	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 5 kg

Catalog #	Description
Foresight Prepacked Columns	
732-4735	**Foresight CHT Type I Column**, 40 µm, 1 ml
732-4755	**Foresight CHT Type I Column**, 40 µm, 5 ml
732-4736	**Foresight CHT Type II Column**, 40 µm, 1 ml
732-4756	**Foresight CHT Type II Column**, 40 µm, 5 ml
Foresight Prepacked Chromatography Filter Plates*	
732-4716	**Foresight CHT Type I Plates**, 40 µm, 20 µl
732-4718	**Foresight CHT Type II Plates**, 40 µm, 20 µl
Foresight RoboColumn Units**	
732-4822	**Foresight CHT Type I RoboColumn Units**, 40 µm, 200 µl
732-4823	**Foresight CHT Type I RoboColumn Units**, 40 µm, 600 µl
732-4825	**Foresight CHT Type II RoboColumn Units**, 40 µm, 200 µl
732-4826	**Foresight CHT Type II RoboColumn Units**, 40 µm, 600 µl
CFT Ceramic Fluoroapatite Media, Type II	
1585200	**CFT Ceramic Fluoroapatite**, 40 µm, Type II, 10 g
1575000	**CFT Ceramic Fluoroapatite**, 40 µm, Type II, 100 g
157-5100	**CFT Ceramic Fluoroapatite**, 40 µm, Type II, 1 kg
157-5500	**CFT Ceramic Fluoroapatite**, 40 µm, Type II, 5 kg
MPC Ceramic Hydroxyfluoroapatite Media, Type I	
1580200	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 10 g
1570200	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 100 g
157-0201	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 1 kg
157-0205	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 5 kg
Foresight Prepacked Columns	
732-4737	**Foresight MPC Type I Column**, 40 µm, 1 ml
732-4757	**Foresight MPC Type I Column**, 40 µm, 5 ml
Foresight Prepacked Chromatography Filter Plates*	
732-4785	**Foresight MPC Type I Plates**, 40 µm, 20 µl
Foresight RoboColumn Units**	
732-4828	**Foresight MPC Type I RoboColumn Units**, 40 µm, 200 µl
732-4829	**Foresight CHT Type I RoboColumn Units**, 40 µm, 600 µl

* Package size: 2 x 96-well plates.
** Package size: one row of eight columns.

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