
EconoFit CHT Type I, CHT Type II, CHT XT, MPC, and CFT Columns, 1 and 5 ml

Instruction Manual

Catalog number

12009255
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12009240
12009251

Please read the instructions in this manual prior to using EconoFit Columns. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Section 1

Introduction

EconoFit Ceramic Apatite Columns are convenient, disposable, prepacked low-pressure chromatography columns. EconoFit Columns offer both increased run-to-run uniformity and high purity of proteins through the column design and novel resin technology. Compatible with most aqueous buffers commonly used for protein purification, they offer improved performance for your protein separation needs and are supplied ready for use in convenient 1 and 5 ml sizes. They can be quickly connected to liquid chromatography systems using 10-32 fittings.

Section 2

Product Information

CHT Ceramic Hydroxyapatite Media is a spherical, macroporous form of hydroxyapatite. It has been sintered at high temperatures to modify it from a nanocrystalline to a ceramic form. The ceramic material retains the unique separation properties of crystalline hydroxyapatite. Unlike most other chromatography adsorbents, CHT Media is both the ligand and the support matrix. Separation protocols originally developed on crystalline hydroxyapatite can often be transferred directly to the ceramic material with only minor modifications.

CHT Ceramic Hydroxyapatite Media

CHT Ceramic Hydroxyapatite Media ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) Types I and II EconoFit Columns are available in two particle sizes, 40 and 80 μm . CHT Ceramic Hydroxyapatite XT Media EconoFit Columns are available in the 40 μm particle size. CHT Type I has the highest overall protein binding capacity among the three varieties. It also has the best capacity for acidic proteins in general. CHT XT is the newest member of the CHT family. It is similar in application usage to CHT Type I and is a more stable matrix, providing longer usable column life at process scale. CHT Type II, with its large pore size, is the most effective option for purification of large biomolecules such as IgM and viruses.

MPC Ceramic Hydroxyfluoroapatite Media

MPC Ceramic Hydroxyfluoroapatite Media ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{1.5}(\text{F})_{0.5}$) is a complementary addition to our line of ceramic apatite mixed-mode chromatography media. MPC is a composite of hydroxyapatite and fluoroapatite and retains the unique separation properties and unmatched selectivity and resolution of our CHT Ceramic Hydroxyapatite Media.

CFT Ceramic Fluoroapatite Media

CFT Ceramic Fluoroapatite Media ($\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$) is a rigid spherical macroporous media. CFT is a composite of fluoroapatite and hydroxyapatite prepared by chemically converting hydroxyapatite nanocrystals to fluoroapatite with a fluorine reagent. CFT can be used under stringent chromatography requirements to separate acidic proteins requiring buffered conditions as low as pH 5.6.

Chromatography media properties.

	CHT			MPC	CFT
	Type I	Type II	XT	Type I	Type II
Functional groups	Ca ²⁺ , PO ₄ , OH	Ca ²⁺ , PO ₄ , OH	Ca ²⁺ , PO ₄ , OH	Ca ²⁺ , PO ₄ , OH, F	Ca ²⁺ , PO ₄ , F
Observed dynamic binding capacity lysozyme (Lys)	25–40 mg Lys/g CHT	12.5–20 mg Lys/g CHT	17.4–24.8 mg Lys/g CHT	25–40 mg Lys/g MPC	14–21.5 mg Lys/g CFT
Mean particle size	20 ± 2, 40 ± 4, and 80 ± 8 µm	20 ± 2, 40 ± 4, and 80 ± 8 µm	40 ± 4 µm	40 ± 4 µm	40
Tap-settled density* (g/ml tap settled bed)	0.63 g/ml	0.63 g/ml	0.67 g/ml**	0.72 g/ml	0.86 g/ml

* Under ideal conditions.

** Determined from preliminary data.

All ceramic apatites are also available in larger bottle sizes. Please refer to the ordering section for more information.

Section 3

Method Development

The following protocols have been developed as general starting guidelines for the purification of most proteins and nucleic acids and may help to reduce time spent in method development.

General protocol: phosphate elution.*

Flow rate	150 cm/hr for all steps (1 ml/min for 1 ml column; 5 ml/min for 5 ml column)
Wash	H ₂ O
Pre-equilibrate	400 mM NaPO ₄ , pH 6.8
Equilibrate	5 mM NaPO ₄ , 20 ppm Ca ⁺⁺ , 150 mM NaCl, pH 6.8
Load	Clarified sample: 5 mM NaPO ₄ , 20 ppm Ca ⁺⁺ , 150 mM NaCl, pH 6.8
Wash	5 mM NaPO ₄ , 20 ppm Ca ⁺⁺ , 150 mM NaCl, pH 6.8
Elute	Linear gradient: 5 mM NaPO ₄ + 20 ppm Ca ⁺⁺ – 500 mM NaPO ₄ , 12 ppm Ca ⁺⁺ , 150 mM NaCl, pH 6.8
Clean	400 mM NaPO ₄ , pH 6.8
Wash	H ₂ O
Sanitize	1.0 N NaOH

* Please refer to bio-rad.com/chtguide or bulletin 6086, Development Guidelines, for additional method development information.

Sample load should be free of agents such as citrate or EDTA that could degrade CHT Ceramic Hydroxyapatite via chelation. CHT is chemically compatible with the following solutions at pH 6.5–14 in the presence of calcium and phosphate.

- 2 N NaOH*
- 6 M guanidine-HCl
- 8 M urea
- 100% acetonitrile
- 100% ethanol
- 1% SDS and other surfactants (not calcium compatible)
- 4 M NaCl
- 1 M potassium phosphate**
- 0.5 M sodium phosphate**

* No Ca or PO₄ required.

** No Ca required.

Section 4

Regenerating, Sanitizing, and Storing Columns

Regeneration

CHT Ceramic Hydroxyapatite Columns should be regenerated at the completion of each run with 3–5 column volumes (CV) of 400 mM sodium phosphate, pH 7.0–7.5, or 400 mM trisodium phosphate, pH 11–12. If higher concentrations of phosphate are needed to remove tightly bound species, use potassium phosphate. The column can also be stripped with other cleaning solutions (1–2 M KCl or NaCl, 8 M urea, or 6 M guanidine-HCl) containing 5 mM phosphate at neutral pH. Note that sodium phosphate at pH 6.8 was used in the general protocol.

Sanitization

The column can be sanitized in up to 2 N NaOH. Owing to the higher viscosity of concentrated NaOH solutions, the flow rate may need to be lowered to avoid overpressure issues.

Storage

CHT Columns can be stored at room temperature in 0.1 N NaOH.

Section 5

Scaling Up

EconoFit Ceramic Apatite Columns are available in 1 and 5 ml formats. The media is also available in various amounts, from 10 gram bottles to larger bulk quantities, for scaling up methods developed using the columns. For quick scale-up, two or three columns of the same type can be connected in series, so take care to maintain an overall system pressure ≤ 72 psi or 5 bar. For additional information on scaling up ceramic apatite media, please visit [bio-rad.com/chtguide](https://www.bio-rad.com/chtguide) or request a copy of bulletin 6086. This is an application guide for process development and scale-up.

Speak to your local Bio-Rad representative or go to [bio-rad.com/ResinsandColumns](https://www.bio-rad.com/ResinsandColumns) for more information on resin and prepacked columns.

Section 6

Troubleshooting Guide

Possible Causes	Possible Solutions
High column pressure	Clogged column screen/frit
Particulates in sample	Filter all samples and buffers through 0.2 µm filter prior to application
Low level of target	Check expression level of protein in starting SDS-PAGE material
Binding capacity of column exceeded	Load less sample

Section 7

Ordering Information

Catalog # Description

CHT Ceramic Hydroxyapatite Type I Media

1582000	CHT Ceramic Hydroxyapatite Type I, 20 µm, 10 g
1570020	CHT Ceramic Hydroxyapatite Type I, 20 µm, 100 g
157-0021	CHT Ceramic Hydroxyapatite Type I, 20 µm, 1 kg (1.6 L)
1584000	CHT Ceramic Hydroxyapatite Type I, 40 µm, 10 g
1570040	CHT Ceramic Hydroxyapatite Type I, 40 µm, 100 g
157-0041	CHT Ceramic Hydroxyapatite Type I, 40 µm, 1 kg (1.6 L)
157-0045	CHT Ceramic Hydroxyapatite Type I, 40 µm, 5 kg (7.9 L)
1588000	CHT Ceramic Hydroxyapatite Type I, 80 µm, 10 g
1570080	CHT Ceramic Hydroxyapatite Type I, 80 µm, 100 g
157-0081	CHT Ceramic Hydroxyapatite Type I, 80 µm, 1 kg (1.6 L)
157-0085	CHT Ceramic Hydroxyapatite Type I, 80 µm, 5 kg (7.9 L)

CHT Ceramic Hydroxyapatite Type II Media

1582200	CHT Ceramic Hydroxyapatite Type II, 20 µm, 10 g
1572000	CHT Ceramic Hydroxyapatite Type II, 20 µm, 100 g
157-2100	CHT Ceramic Hydroxyapatite Type II, 20 µm, 1 kg (1.6 L)
1584200	CHT Ceramic Hydroxyapatite Type II, 40 µm, 10 g
1574000	CHT Ceramic Hydroxyapatite Type II, 40 µm, 100 g
157-4100	CHT Ceramic Hydroxyapatite Type II, 40 µm, 1 kg (1.6 L)
157-4500	CHT Ceramic Hydroxyapatite Type II, 40 µm, 5 kg (7.9 L)
1588200	CHT Ceramic Hydroxyapatite Type II, 80 µm, 10 g
1578000	CHT Ceramic Hydroxyapatite Type II, 80 µm, 100 g
157-8100	CHT Ceramic Hydroxyapatite Type II, 80 µm, 1 kg (1.6 L)
157-8500	CHT Ceramic Hydroxyapatite Type II, 80 µm, 5 kg (7.9 L)

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 (1.5 L)
12002455	CHT Ceramic Hydroxyapatite XT, 40 µm, 5 kg (7.5 L)

MPC Ceramic Hydroxyfluoroapatite Type I Media

1580200	MPC Ceramic Hydroxyfluoroapatite Type I, 40 µm, 10 g
1570200	MPC Ceramic Hydroxyfluoroapatite Type I, 40 µm, 100 g
157-0201	MPC Ceramic Hydroxyfluoroapatite Type I, 40 µm, 1 kg (1.4 L)
157-0205	MPC Ceramic Hydroxyfluoroapatite Type I, 40 µm, 5 kg (6.9 L)

Catalog #	Description
CFT Ceramic Fluoroapatite Type II Media	
1585200	CFT Ceramic Fluoroapatite Type II, 40 µm, 10 g
1575000	CFT Ceramic Fluoroapatite Type II, 40 µm, 100 g
157-5100	CFT Ceramic Fluoroapatite Type II, 40 µm, 1 kg (1.2 L)
157-5500	CFT Ceramic Fluoroapatite Type II, 40 µm, 5 kg (5.8 L)

Foresight Columns

732-4737	Foresight MPC Type I Column, 40 µm, 1 ml
732-4757	Foresight MPC Type I Column, 40 µm, 5 ml
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
12003150	Foresight CHT XT Column, 40 µm, 1 ml
12003149	Foresight CHT XT Column, 40 µm, 5 ml

Foresight Plates*

732-4785	Foresight MPC Type I Plates, 40 µm, 20 µl
732-4716	Foresight CHT Type I Plates, 40 µm, 20 µl
732-4718	Foresight CHT Type II Plates, 40 µm, 20 µl
12003151	Foresight CHT XT Plates, 40 µm, 20 µl

* Package size: 2 x 96-well plates.

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
732-4828	Foresight MPC Type I RoboColumn Units, 40 µm, 200 µl
732-4829	Foresight MPC Type I RoboColumn Units, 40 µm, 600 µl
12003152	Foresight CHT XT RoboColumn Units, 40 µm, 200 µl
12003148	Foresight CHT XT RoboColumn Units, 40 µm, 600 µl

** Package size: 1 row of 8 columns

EconoFit Columns

12009255	EconoFit CHT Type I Column, 40 µm, 1 x 1 ml, 7 x 25 mm
12009253	EconoFit CHT Type I Column, 40 µm, 1 x 5 ml, 16 x 25 mm
12009254	EconoFit CHT Type I Columns, 40 µm, 5 x 5 ml, 16 x 25 mm
12009256	EconoFit CHT Type I Column, 80 µm, 1 x 1 ml, 7 x 25 mm
12009259	EconoFit CHT Type II Column, 40 µm, 1 x 1 ml, 7 x 25 mm
12009257	EconoFit CHT Type II Column, 40 µm, 1 x 5 ml, 16 x 25 mm
12009258	EconoFit CHT Type II Columns, 40 µm, 5 x 5 ml, 16 x 25 mm
12009260	EconoFit CHT Type II Column, 80 µm, 1 x 1 ml, 7 x 25 mm
12009261	EconoFit CHT XT Column, 1 x 1 ml, 7 x 25 mm
12009279	EconoFit MPC I Column, 1 x 1 ml, 7 x 25 mm
12009252	EconoFit CFT Type II Column, 40 µm, 1 x 1 ml, 7 x 25 mm
12009240	EconoFit CFT Type II Column, 40 µm, 1 x 5 ml, 16 x 25 mm
12009251	EconoFit CFT Type II Columns, 40 µm, 5 x 5 ml, 16 x 25 mm

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Larger volumes and special packaging are available upon request.

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