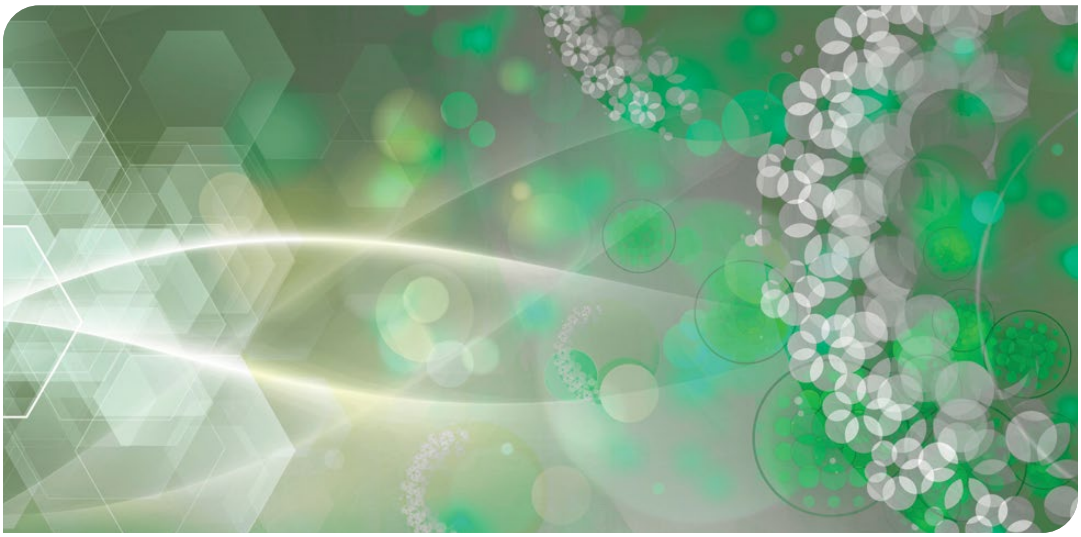


Mixed-Mode Chromatography: Ceramic Apatite Media

Introduction to CHT Ceramic Hydroxyapatite

CHT Ceramic Hydroxyapatite is a mixed-mode chromatography media with unique separation properties and unmatched selectivity and resolution. It is used for the purification of a variety of biomolecules.

Matrix material	▪ Hydroxyapatite is a crystalline mineral of calcium and phosphate ▪ The ligand and the matrix are the same ▪ Formula: $Ca_{10}(PO_4)_6(OH)_2$
Available functionalities	▪ Mixed mode ▪ Offers cation exchange and calcium affinity interactions
Most popular applications	▪ Monoclonal antibody purification ▪ Vaccine manufacturing ▪ Removal of aggregate/process impurities
Additional media types	▪ CFT Ceramic Fluoroapatite ▪ MPC Ceramic Hydroxyfluoroapatite

BIO-RAD

Ceramic Apatite Product Line

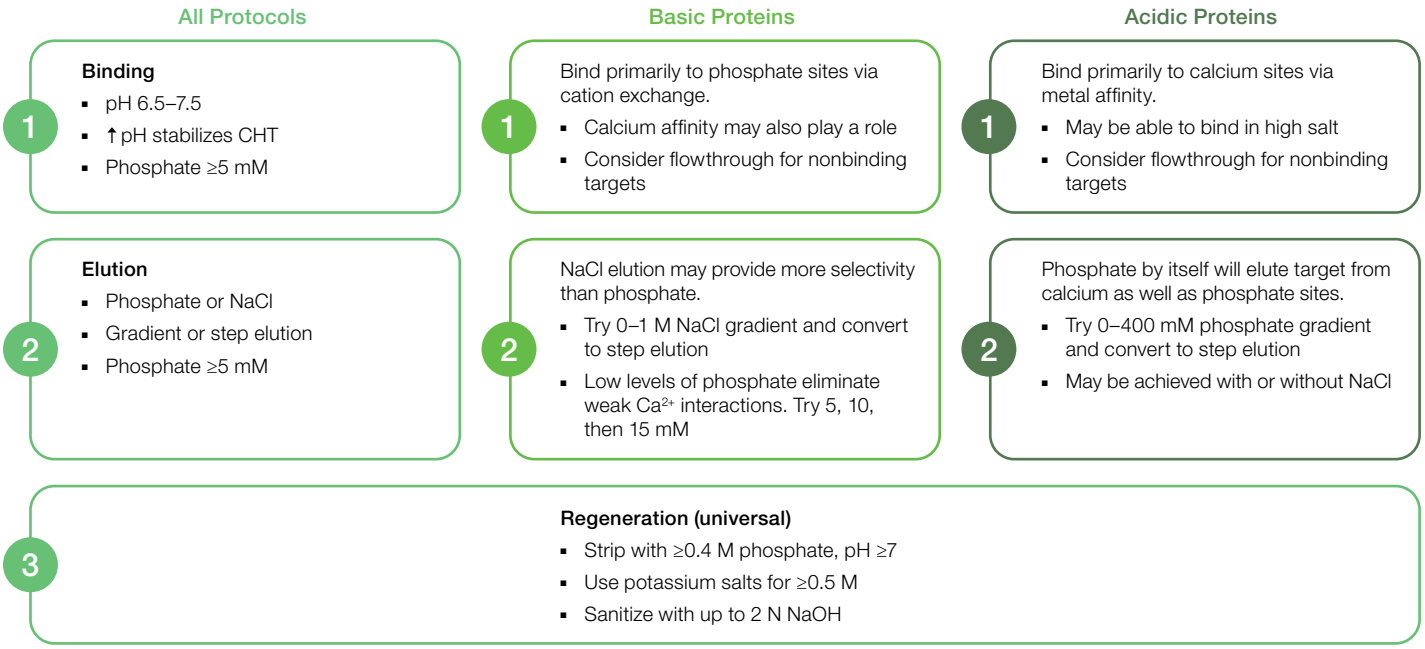
CHT is available in three types (Type I, Type II, and XT) and different particle sizes.* This offers the flexibility to select the most suitable media for a given application. In addition, two other ceramic apatites are available, CFT and MPC.

Product	Attributes
CHT Type I, 40 µm	▪ Particle size 40 µm ▪ Smallest pore size among the CHT types (same pore size as Type I, 80 µm) ▪ Highest dynamic binding capacity (DBC) due to the largest surface area among the CHT types ▪ Binds molecules in the broadest range of pI values ▪ Strongest binding to acidic molecules among all CHT types
CHT Type I, 80 µm	▪ Particle size 80 µm ▪ Smallest pore size among the CHT types (same pore size as Type I, 40 um) ▪ Highest DBC due to the largest surface area among the CHT types. May require slower flow to achieve similar DBC to Type I, 40 µm. Pore size is same as Type I, 40 µm, but mass transfer may be less efficient at faster flow due to the larger particle size
CHT Type II, 40 µm	▪ Particle size 40 µm ▪ Largest pore size among the CHT types (same pore size as Type II, 80 µm) ▪ Lowest DBC due to the smallest surface area among the CHT types ▪ Binds well to molecules of neutral and basic nature (similar to MPC and CHT XT)
CHT Type II, 80 µm	▪ Particle size 80 µm ▪ Largest pore size among the CHT types (same pore size as Type II, 40 µm) ▪ Lowest DBC due to the smallest surface area among the CHT types ▪ Enables faster flow rates due to the larger particle size
CHT XT, 40 µm	▪ Particle size 40 µm ▪ Robust matrix providing longer usable column life compared to Type I, 40 µm, at process scale ▪ Pore size is slightly larger than Type I and smaller than Type II ▪ Selectivity is between Type I and Type II ▪ Binds well to molecules of neutral and basic nature (similar to MPC and Type II)
CFT Type II, 40 µm	▪ Particle size 40 µm ▪ Ceramic fluoroapatite ▪ More stable than CHT under acidic conditions, down to pH 5.6
MPC Type I, 40 µm	▪ Particle size 40 µm ▪ A fluorinated derivative of ceramic hydroxyapatite with increased matrix stability for specialty applications ▪ CHT XT or Type I are recommended as a first option

* CHT Types I and II are also available in a 20 µm particle size.

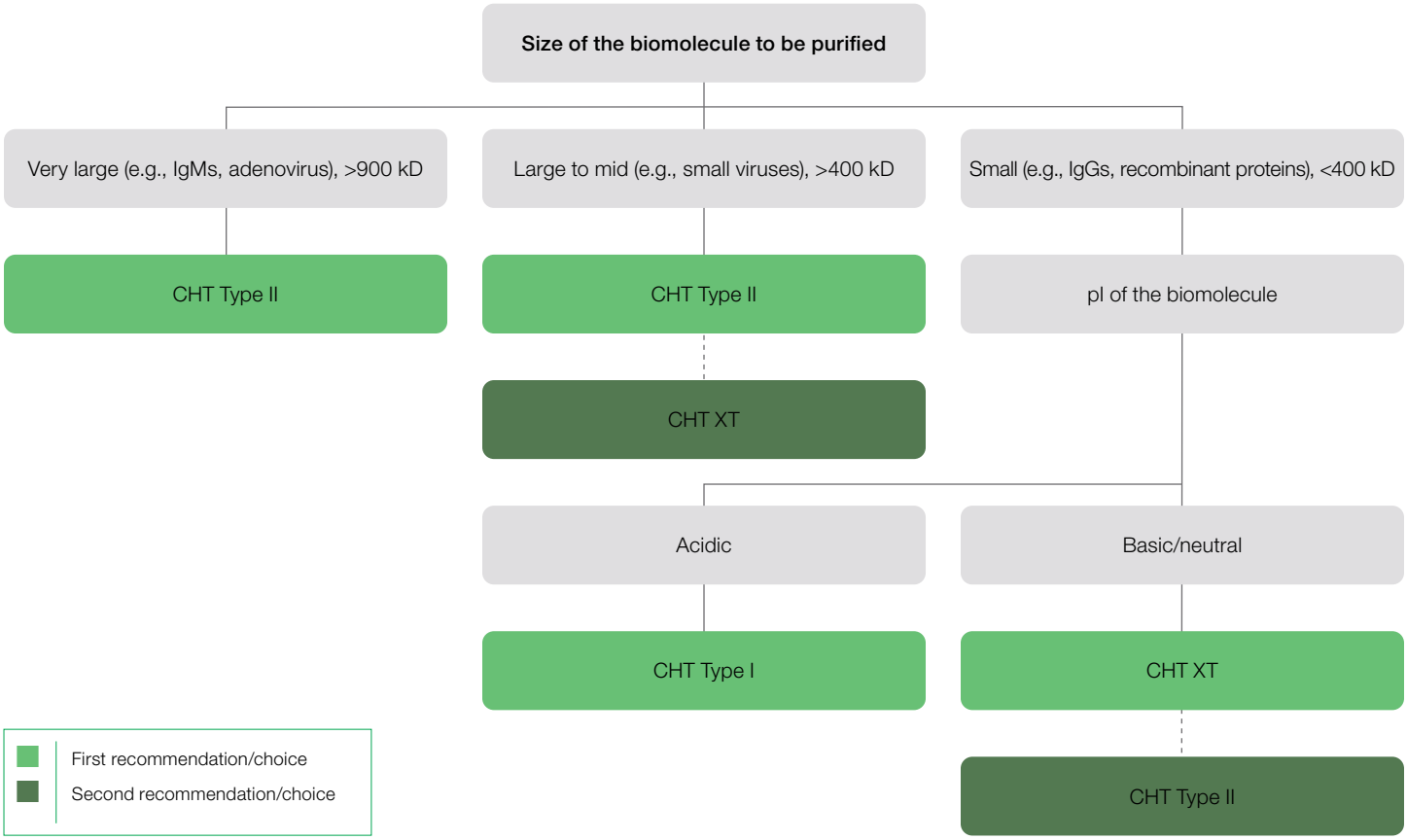
General Guidelines for Method Development with CHT

Initial scouting protocols under bind and elute conditions can be suggested depending on the pI of the protein or biomolecule of interest.



CHT Selection Guidelines

Choosing the appropriate CHT depends on multiple criteria, as shown in the selection tree. Use the following chart to select the CHT Media that is suitable for your application.



Best Practices for CHT Use

- Use hydrated buffer salts
  - Do not use anhydrous sodium phosphate or dodecahydrates as these 2 salt types can cause irreproducible results
- Avoid ethylenediaminetetraacetic acid and other chelating agents
- When packing your own columns, do not compress CHT
  - CHT is an incompressible media like silica or controlled-pore glass
  - Upflow after packing is not recommended

Bio-Rad Mixed-Mode Offering in Prepacked EconoFit Columns

Prepacked and disposable EconoFit Columns offer a convenient way to incorporate mixed-mode chromatography in your purification workflow, either at laboratory scale or just for early method development.

Catalog numbers by column size and configuration are shown.

	Catalog Numbers by Column Size		
Media	**1 x 1 ml**	**1 x 5 ml**	**5 x 5 ml**
CHT Type I, 40 µm	12009255	12009253	12009254
CHT Type II, 40 µm	12009259	12009257	12009258
CHT Type I, 80 µm	12009256	–	–
CHT Type II, 80 µm	12009260	–	–
CHT XT, 40 µm	12009261	–	–
CFT Type II, 40 µm	12009252	12009240	12009251
MPC Type I, 40 µm	12009279	–	–

Additional Information

Visit bio-rad.com/CHTGuide to download our ceramic hydroxyapatite application guide.

Visit bio-rad.com/EconoFit to view the complete EconoFit product line offering.

Visit bio-rad.com/MixedMode to view our bulk media offering.

EconoFit Columns: Prepacked, disposable, low-pressure columns that are compatible with commonly used chromatography systems.

Bio-Rad Laboratories, Inc.

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