

Good Practices for CHT™

Ceramic Hydroxyapatite Media Usage

THE GOLD STANDARD

CHT Ceramic Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is an easy-to-use mixed-mode chromatography media. It offers cation exchange and affinity interactions. Unlike other chromatography media, CHT is both the ligand and the support matrix. Two types of CHT, Type I and Type II, are available in three particle sizes — 20, 40, and 80 μm . It is used for the purification of numerous types of biomolecules and is considered **the gold standard for aggregate removal**. In this wall chart, we list guidelines for the proper use of CHT in biomolecule purification.

What Can CHT Purify?

- Monoclonal and polyclonal antibodies and antibody fragments
- Antibodies that differ in light chain composition
- Recombinant proteins and isozymes
- Viruses, viral particles, and vaccines
- Supercoiled DNA from linear duplexes
- Single-stranded from double-stranded DNA

Golden Rules for CHT Use

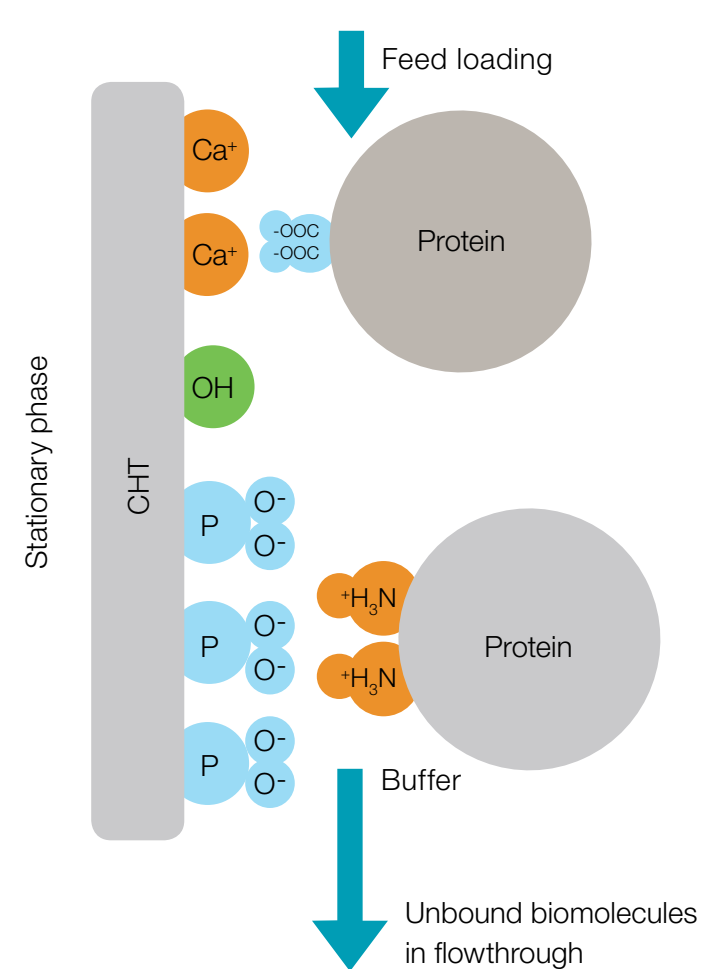
- Do not compress
- Keep pH >6.5 throughout purification
- Add phosphate and calcium to all buffers (unless otherwise noted)

CHT Packing

- CHT is composed of incompressible particles, so avoid compression during packing
- Compatible with axial (without compression), flow, and pressure packing
- Upflow after packing is not recommended

Binding – Chemical Compatibility

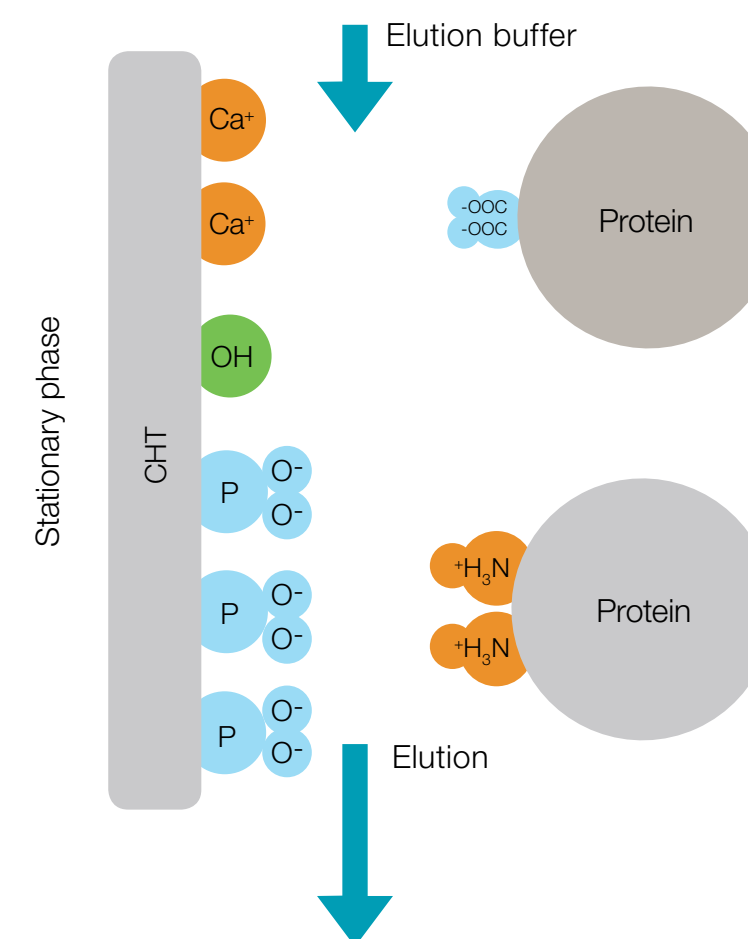
Optimal binding of biomolecules on CHT depends on the feedstock/buffer components.



- ✓ Commonly used binding buffers**
Phosphate-, Tris-, HEPES-, and MES-based buffers containing CHT compatible chemicals, such as:
- Guanidine-HCl
 - Urea
 - Acetonitrile
 - Ethanol/methanol
 - SDS and other surfactants
 - Neutral salts
 - Glycine
- ✗ Chemicals not recommended in the binding buffer**
- EDTA, citrate, and other chelating agents

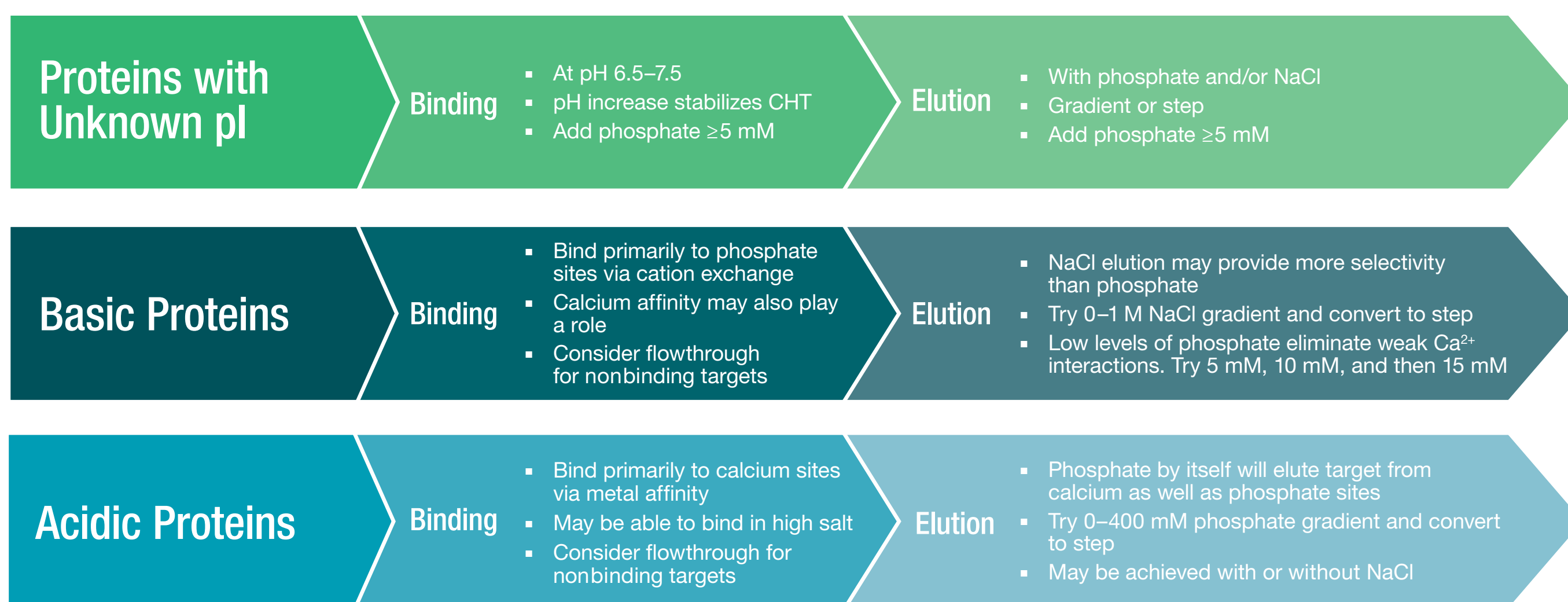
Elution

Elutions can be performed with phosphate-based buffers to desorb metal affinity interactions and/or with NaCl-based buffers to desorb cation exchange interactions.

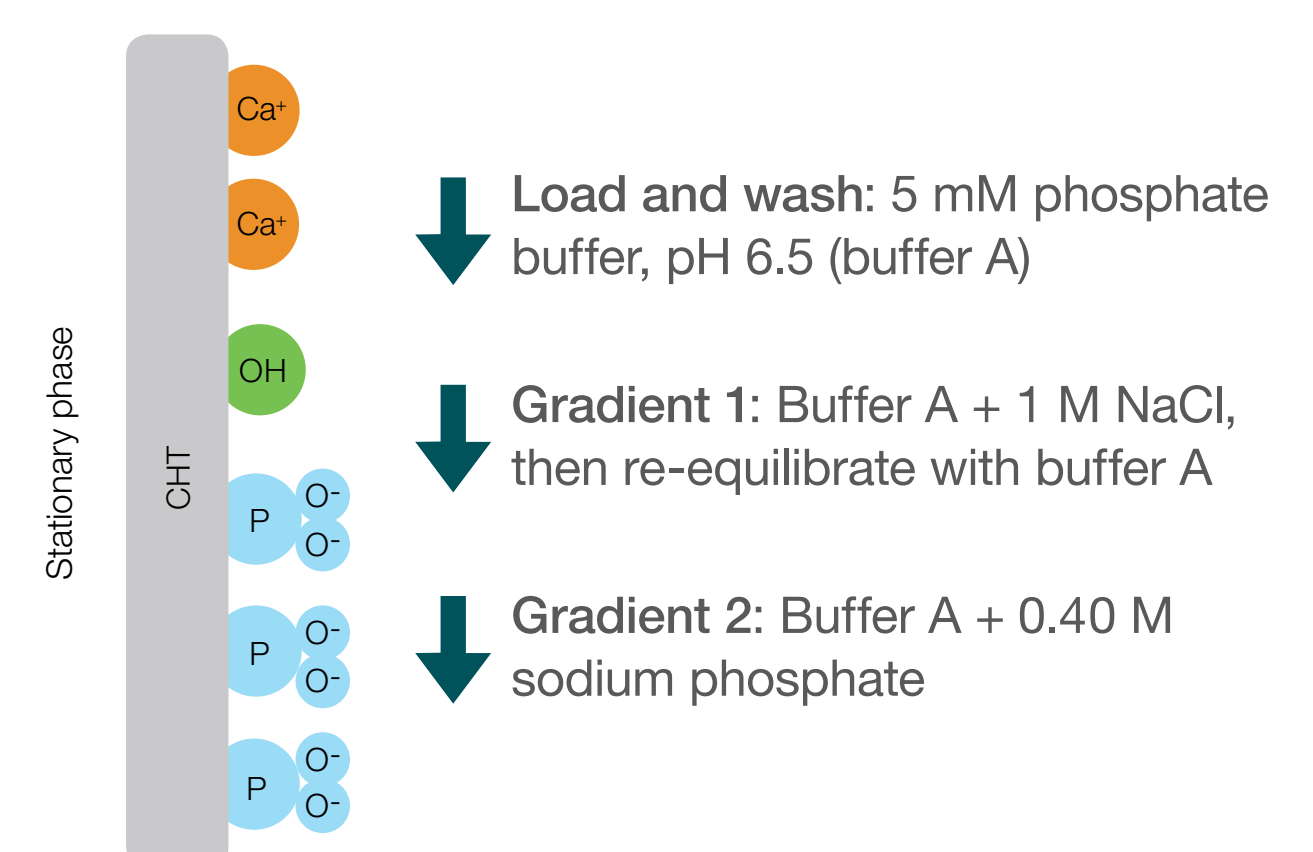


- ✓ Commonly used elution buffers**
- Acidic proteins and small DNA fragments: phosphate gradient
 - Basic proteins: salt or pH gradient
 - Mammalian viruses: up to 600 mM phosphate buffer
 - Chromosomal DNA: 0.2–0.4 M phosphate buffer (higher if NaCl is present)
- Some conditions will cause a drop in pH.¹

General Guidelines for Method Development



Example Method



Regeneration

- Regenerate with 3–5 CV of 500 mM potassium or sodium phosphate buffer at \sim pH 7.5 or 400 mM trisodium phosphate at pH 11–12

Storage

- Store unused CHT in the original container at ambient temperature
- Store regenerated and sanitized CHT in 0.1–1.0 N NaOH at or below ambient temperature

Sanitization

- CHT Columns can be sanitized in up to 2 N NaOH and stored in 0.1–1.0 N NaOH

¹ Importance of maintaining pH >6.5: increasing the solution concentration of cations displaces protons bound to CHT phosphate groups into solution, which can lead to a drop in pH. Exposure to acidic conditions (pH <6.5) can have a deleterious effect on the lifetime of CHT.

Changes in pH can be minimized by:

- Using co-buffers such as MES, MOPS, PIPES, HEPES, ACES, MOPSO, Tris, histidine, lysine, and arginine
- Performing a surface neutralization right before elution. Typically a wash with 7–8 CV 25 mM Tris, 25 mM NaCl, 5 mM phosphate at pH 7.75
- Using hydrated buffer salts. Do not use anhydrous sodium phosphate or dodecahydrates as these two salt types can cause irreproducible results.

BIO-RAD

Visit bio-rad.com/ProcessResins to get detailed information about CHT Ceramic Hydroxyapatite.
Visit bio-rad.com/ProteinPurification to learn about the different applications for CHT Ceramic Hydroxyapatite.

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