How Does CHT™ Ceramic Hydroxyapatite Media Work?

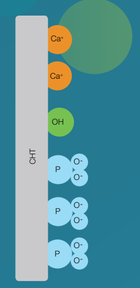
CHT Chemical Interactions

CHT Structure

CHT Ceramic Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is a mixed-mode chromatography media used for the purification of monoclonal and polyclonal antibodies, antibody fragments, enzymes, nucleic acids, and membrane proteins. It is formed from the chemical combination of calcium and phosphate salts. Biomolecules can interact with CHT through calcium affinity interactions and/or cation exchange interactions. This wall chart highlights the different ways in which these chemical interactions can occur.

Each molecule of CHT consists of:

- 5 positively charged calcium pairs (C-sites)
- 2 hydroxyl residues
- 2 phosphate triplets (P-sites), each with 6 negatively charged oxygen atoms



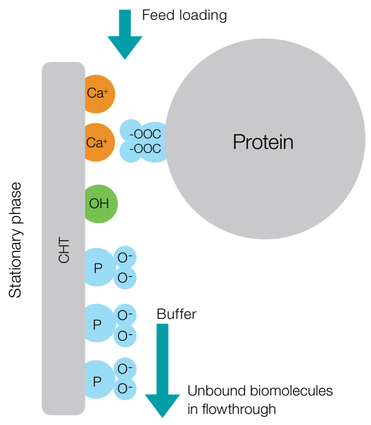
CHT Chemical Interaction Mantra

- Carboxyl groups on biomolecules are attracted to C-sites and repelled by P-sites on CHT
- Amino groups on biomolecules are attracted to P-sites and repelled by C-sites on CHT

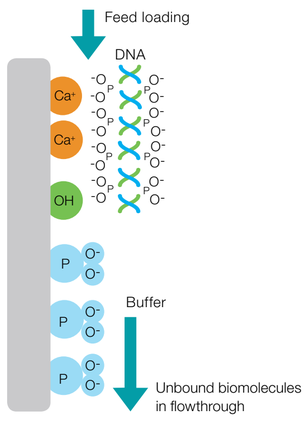
CHT Metal Affinity Interactions

Binding

Interaction of calcium groups on CHT with carboxyl groups on proteins.



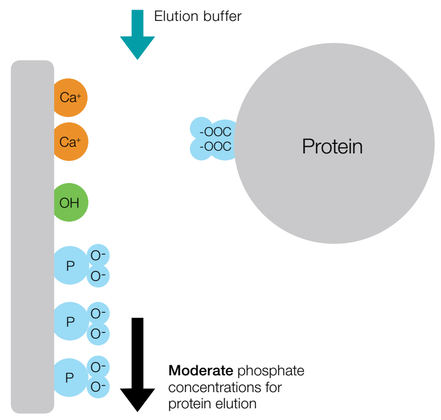
Interaction of calcium groups on CHT with phosphoryl groups on nucleic acids.

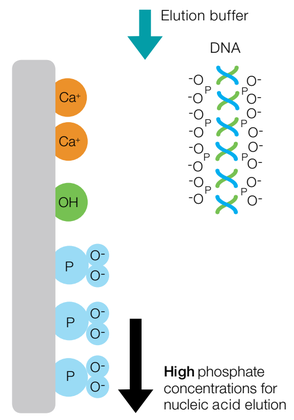


CHT typically interacts with acidic proteins, antibodies, nucleic acids, endotoxins, and enveloped viruses through metal affinity interactions.

Elution

Desorption from metal affinity sites due to increasing phosphate gradient.



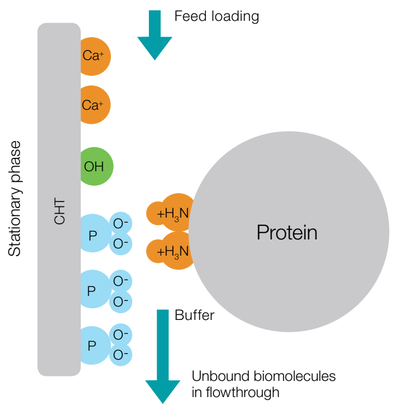


Phosphate gradients are typically used to elute biomolecules bound to CHT by metal affinity interaction. However, NaCl also affects their retention time, indicating a minor contribution by cation exchange.[1]

CHT Cation Exchange Interactions

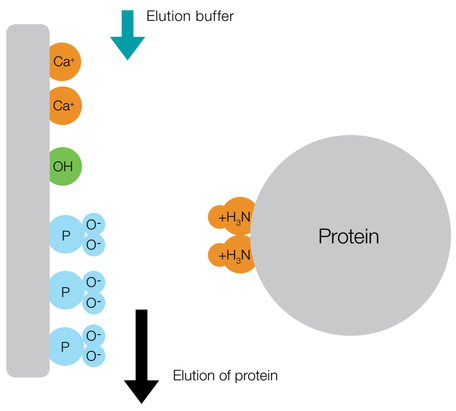
Binding

Interaction of phosphate groups on CHT with amino groups on proteins.



Elution

Desorption from cation exchange sites due to increasing salt gradients or increasing pH.

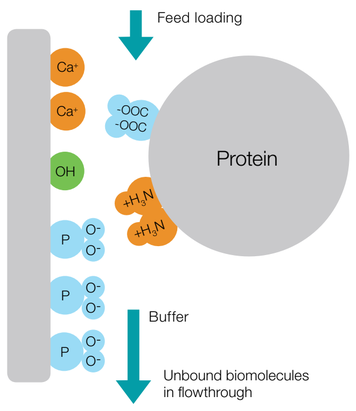


CHT typically interacts with basic proteins and antibodies through cation exchange interactions. Salt or pH gradients are usually used to elute these bound biomolecules.

CHT Mixed-Mode Interactions

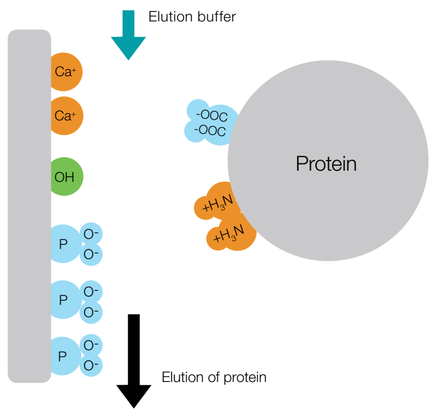
Binding

Binding of proteins to CHT through a combination of affinity and cation exchange interactions.



Elution

Desorption from metal affinity and cation exchange sites due to increasing NaCl concentrations in the presence of low levels (5–25 mM) of phosphates.[2]



CHT typically interacts with monoclonal antibodies through mixed-mode interactions. A combination of phosphate and NaCl is usually required to elute these bound biomolecules.

CHT metal affinity interactions are 15 to 60 times stronger than ionic interactions and are not affected by increasing ionic strength when using typical elution ions.

Tips and Tricks

Load Preparation: Initial load should be free of agents such as citrate or EDTA that could degrade CHT via chelation.
Purification Process: Keep pH >6.5 during purification. For increased CHT stability, phosphate levels should be at least 5 mM in process solutions. Use hydrated buffer salts. Do not use anhydrous sodium phosphate or dodecahydrates. These two salt types can cause irreproducible results.

[1] NaCl at 1.0 M typically reduces retention time by approximately 10% in the presence of phosphate gradients, indicating a minor contribution by cation exchange.
[2] Unless a minimal concentration of phosphate is present, most IgGs remain bound to CHT even in saturated NaCl. Retention on CHT is progressively reduced with increased phosphate concentration.

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Visit bio-rad.com/web/CHTwallchart to get detailed information about CHT Ceramic Hydroxyapatite and bio-rad.com/web/ProcessApplications to learn about its various applications.

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