Hydroxyapatite Magnetic Beads

Hydroxyapatite chromatography is one of the best-characterized multimodal chromatographic media, with considerable scientific and industrial applications in the field of bioprocessing. Its unique mix of cation exchange and calcium-affinity mechanisms has enabled it to accomplish separations that no other chromatographic media can equal. It continues to bring novel solutions for growing issues in the industry.

Hydroxyapatite is a naturally occurring crystalline substance having the structural formula $Ca_5(PO_4)_3OH$. Its formula gives the impression that calcium residues are the primary characteristic of the crystal surfaces, yet most are involved in subsurface structure maintenance. It leaves phosphate residues as the dominating surface characteristic. Surface ratios average closer to 5:3 Ca:P than the expected 5:3 Ca:P ratio.

Due to its unique mixed-mode ion exchange characteristics, hydroxyapatite has unique separation properties, unparalleled selectivity and resolution, and a tremendous binding capacity for proteins and antibodies. Hydroxyapatite has two adsorbing sites: a calcium site that binds with the acidic groups and a phosphate site that interacts with basic protein groups. Furthermore, hydroxyapatite does not bind free amino acids or small peptides, making it eminently advantageous for protein purification.

BcMag™ Hydroxyapatite Magnetic Beads are uniform magnetic resins coated with hydroxyapatite functional groups on the surface (Fig.1). The hydroxyapatite magnetic resins enable rapid high-yield processing of 96 samples. in about 20 minutes. The hydroxyapatite magnetic beads can quickly purify antibodies, nucleic acids, viruses, and other macromolecules from complex biological samples manually or automatically.

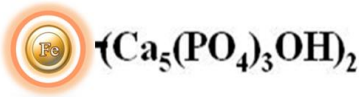

Fig.1 Structure of hydroxyapatite

The hydroxyapatite magnetic beads replace traditional chromatographic matrices such as agarose, cellulose, Sepharose, and Sephadex-based columns or resins for more efficiently processing. In column-based procedures, the lysate is centrifuged or cleared, the supernatant is added to the column, the membrane or resin is washed with buffer through centrifugation or vacuum manifold, and the required biomolecules are eluted in an adequate volume of buffer. When using column-based technologies, processing multiple samples in academic research labs may necessitate a significant quantity of hand pipetting. This pipetting can discourage differences in target biomolecule yield between experiments and people. Staff and students may require extensive training and practice to produce constant protein yields.

The hydroxyapatite resins have significant advantages over non-magnetic resin technologies. It is due to the numerous benefits of magnetic resins, such as their ease of use, rapid experimental protocols, suitability, and convenience for high throughput automated and miniaturized processing. They thus see increasing use in various areas of life-sciences research and development, including drug discovery, biomedicine, bioassay development, diagnostics, genomics, and proteomics.

Feature and benefits:

- **Fast and s**traightforward— Magnetic beads-based format eliminates columns or filters or a laborious repeat of pipetting or centrifugation.
- **Convenient and expandable**—Magnetic format enables high-throughput processing of multiple samples in parallel with many different automated liquid handling systems.
- **Robust**—Magnetic resins do not crack or run dry.
- **Low bed volume**—Working with small magnetic bead volumes allows for minimal buffer volumes, resulting in concentrated elution fractions.
- **Chemical and thermal stability** — A wide range of chemical compatibilities (aqueous and inorganic solvents), heat stability (autoclavable), and pH tolerance (pH >5.5) let hydroxyapatite be utilized below settings that improve nucleic acid and protein binding.

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Specificities	
Composition	Magnetic beads grafted with hydroxyapatite group.
Beads Size	~ 1.0 µm diameter
Magnetization	~45 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.0 g/ml
Stability	Most organic solvents
Formulation	Lyophilized Powder
Binding Capacity	>25µg BSA/ mg of Beads
Storage	Store at 4°C upon receipt

Protocol

Note:

The following protocol is an example of purifying proteins with BcMag™ hydroxyapatite-modified magnetic beads. Users may use alternative binding, washing, or elution buffers and are encouraged to determine the optimal working conditions based on the protocol and suggestions described in the Note sections. We recommend optimizing the amounts of beads used for each application. Adjust elution volumes to avoid unnecessary sample dilution.

Materials Required

- **Magnetic rack (for manual operation):** Based on sample volume, the user can choose one of the following magnetic Racks: BcMag™ magnetic rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag™ magnetic rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag™ magnetic rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag™ magnetic rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04); BcMag™ magnetic rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05).

A. Protein purification

Note:

- Monobasic sodium phosphate monohydrate and dibasic sodium phosphate 7-hydrate are recommended for Precondition Buffer and Binding/Wash buffer preparations. Avoid anhydrous sodium phosphate because these salts contain pyrophosphate that prevents the binding of some macromolecules.
- Avoid High concentrations of salt or chelating agents such as EDTA because they will prevent proteins from binding to the magnetic beads.
- Calcium chloride may be added to the phosphate buffer to increase the binding efficiency of acidic proteins. Concentrations of calcium chloride in phosphate buffers: 0.3 mM calcium chloride for 10 mM phosphate buffers: 0.01 mM calcium chloride for 300 mM phosphate, and 0.0075 mM calcium chloride for 400 mM phosphate.

Buffers

- **Precondition Buffer:** 200 mM Sodium phosphate, pH 9-10
- **Binding/Wash Buffer:** 10 mM Sodium phosphate, pH 6.8, 0.3 mM calcium chloride
- **Elution Buffer:** Gradient of increasing concentration of 10-600 mM potassium phosphate, pH 7.0

a. Sample preparation

1. Dialyze the protein sample against 50 volumes of Binding buffer.

b. Magnetic Beads Preparation

1. Shake the bottle and completely resuspend the Magnetic Beads.
2. Transfer 40µl magnetic beads (2 mg) to a centrifuge tube. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack.
3. Remove the tube and resuspend the beads thoroughly with 200µl Precondition Buffer. Leave the tube at room temperature for 2-3 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack.
4. Repeat step 3 once.

5. Remove the tube from the magnetic rack and resuspend the beads thoroughly with a 200µl Binding/Wash Buffer. Place the tube on a magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack.
6. Repeat step 5 once.
7. Resuspend the beads thoroughly with 100 µl Binding/Wash Buffer.

c. Sample Binding

1. Add your desired sample containing ~40µg protein to the tube containing the washed beads from step B.7.
2. Mix beads well with a pipette and leaves them at room temperature for 2-3 minutes. Place the tube on a magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack.
3. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack. Remove the tube from the rack and wash the beads with a 200µl Binding/Wash Buffer.
4. Repeat step 3 for three times.

d. Proteins Elution

Note:

Protein can be eluted with a gradient of increasing phosphate buffer concentration (10-600 mM) and pH gradient (5.5 or higher, up to the stability limit of the sample protein), or NaCl for basic proteins, but not for acidic proteins.

1. Remove the tube from the magnetic rack. Resuspend the beads with 10-20 µl Elution Buffer and leave them at room temperature for 3 minutes.
2. Place the tube on the magnetic rack for 1-3 minutes and transfer the supernatant containing the eluted protein to a new tube.

B. DNA purification

Buffer

Note: Monobasic sodium phosphate monohydrate and dibasic sodium phosphate 7-hydrate are recommended for all buffer preparations. Avoid anhydrous sodium phosphate because these salts contain pyrophosphate that prevents the binding of some macromolecules.

- **Sample lysis Buffer:** 8M Guanidine hydrochloride
- **Binding/Washing Buffer:** 100 mM phosphate buffer pH 7, 4 M guanidine hydrochloride.
- **Elution Buffer:** 0.5M phosphate buffer pH 7 (4M guanidine hydrochloride -optional)
- **Dilution Buffer:** 0.2 M phosphate buffer pH 7

a. Sample Preparation:

Note: Sample pre-treatment is a critical step for successfully purifying high-quality genomic DNA. Different biological samples require different methods to release their genomic DNA from cells. Many cultured cells can be efficiently homogenized in lysis buffer by vortex, while animal/plant tissues, yeasts, and bacteria need a more powerful lysis process. Suggested methods for preparing different samples are listed in the following table.

Sample	Soft Tissue	Hard Tissue	Plant Tissue	Fungi	Yeast	Bacterium
Liquid nitrogen	+	+	+	+		
Frozen grinding		+			+	+
Homogenize	+	+	+	+	+	+
Lysozyme						+
Lyticase, zymolase					+	
Sonication						+
Glass bead grinding					+	+

1. Dissolve 100 mg pre-treated biomass into 1ml of sample lysis buffer. To promote lysis, incubate at 55 °C for 1 hour to overnight (depending on the sample) on a shaker-incubator.

2. Centrifuge at 13,000 rpm for 3 ~ 5 minutes at room temperature to remove cell debris. Transfer the supernatant to a new tube and adjust the concentration of the supernatant to 4 M guanidine HCl with 2x Dilution buffer.

b. Magnetic Beads Preparation

1. Shake the bottle to resuspend the Magnetic Beads completely.
2. Transfer 20µl-30µl magnetic beads (50 mg/ml) to a centrifuge tube. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack.
3. Remove the tube and resuspend the beads thoroughly with 200µl Binding/Washing. Leave the tube at room temperature for 2-3 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack.
4. Repeat step 3 three times.
5. Resuspend the beads thoroughly with 100 µl Binding/Wash Buffer.

c. Sample Binding

1. Mix the sample with the prepared beads and incubate at room temperature for 10-15 minutes with gentle rotation.
2. Place the tube on a magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack.
3. Remove the tube from the magnetic rack and wash the beads with a 200µl Binding/Wash Buffer. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack.
4. Repeat step 3 for three times.

d. DNA Elution

1. Remove the tube from the magnetic rack, resuspend the beads with 10-50 µl Elution Buffer and leave them at room temperature for 3 minutes.
2. Place the tube on the magnetic rack for 1-3 minutes and transfer the supernatant containing the eluted DNA to a new tube.

e. DNA precipitation

1. Add an equal volume of dH_2O to the eluted DNA solution.
2. Add 0.1 volume 5M ammonium acetate and 2.5 volumes of 100 % EtOH to precipitate the DNA for at least 15 minutes (1 hour or longer is preferred) at -20 °C.
3. Centrifuge for 15 mins at 13,000 rpm. Discard the supernatant. Add 2 ml of ice-cold 70 % ethanol to the pellet and flick/agitate the tube to resuspend the pellet.
4. Centrifuge for 15 mins at 13,000 rpm.
5. Resuspend the DNA in an appropriate amount of dH_2O or TE buffer (note: genomic DNA does not dissolve easily, 5 minutes at 65 °C helps to kick-start dissolving. Discard the supernatant. Allow the DNA to air-dry (15 minutes suffices).

Related Products	
Product Name	**Product Name**
DEAE Magnetic Beads	SCX Magnetic Beads
PSA Magnetic Beads	PEI Magnetic Beads
WCX Magnetic Beads	Hydroxyapatite Magnetic Beads
SAX Magnetic Beads	

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