Weak Ion Exchange Magnetic Beads

Bioclone Weak Ion Exchange Magnetic Beads fractionate proteins or nucleic acids using beads-adsorbent technology as a chromatographic matrix. Ion exchange chromatography is commonly used to separate or purify a target molecule from crude biological materials. The molecules are separated based on variations in their accessible surface charges utilizing very light binding and eluting conditions for intact biological activity,

The Weak Ion Exchange magnetic resins replace time-consuming, difficult, and expensive chromatographic techniques such as agarose, cellulose, sepharose, and Sephadex-based columns or resins. In column-based methods, the lysate would be centrifuged or clarified, the supernatant would be added to column, the membrane or resin would be washed with buffer via centrifugation or vacuum manifold, and the desired biomolecules would be eluted in an appropriate 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 result in discouraging differences in target biomolecule yield between experiments and people. To produce reasonably constant biomolecule yields, staff and students may require extensive training and practice.

Magnetic 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.

Ion exchange chromatography (IEX) separates ionizable compounds according to their total charge. Because adjusting buffer pH can easily change the charge on the molecule, this technique allows for separating similar types of molecules that would be hard to separate using other methods. Ion exchange chromatography is a highly effective separation technology for preparative and analytical chromatography. It is widely used to separate and purify many charged or ionizable molecules such as proteins, peptides, enzymes, nucleotides, DNA, antibiotics, vitamins, etc.

BcMag™ Weak Ion Exchange Magnetic Beads are uniform magnetic resins grafted with a high density of a carboxylate functional group (weak cation exchange) or Diethylaminoethyl (DEAE) group (weak anion exchange) on the surface. The Weak Ion Exchange magnetic bead-based format enables rapid high-yield processing of 96 samples in about 20 minutes. It can quickly fraction proteins or nucleic acids from complex biological samples (such as serum, plasma, etc.) manually or automatically. The purified protein can be used in downstream applications such as sample fractionation for 1D and 2D SDS-PAGE, X-ray crystallization, and NMR spectroscopy. The Weak Ion Exchange resins allow the rapid release of very strong ions that may be retained irreversibly on Strong Ion Exchange beads. Additionally, weak ion exchangers can be effective separation tools when strong ion exchangers fail because the selectivities of weak and strong ion exchangers frequently differ.

Feature and benefits:

- **Fast and simple**— 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.

Phone: (858)-909-0079) **Bioclone Inc**. Fax: (858)-909-0057

Applications:

- Protein pre-fractionation in cell lysates
- Optimizing purification conditions for new protein preparation protocols
- Protein purification and concentration
- Antibody purification from serum, ascites, or tissue culture supernatant
- Preparation of samples prior to 1D or 2D PAGE
- Phosphopeptide purification prior to MS analysis

Note:

- For the convenience of usage, each Bioclone Weak Ion Exchange Bead is labeled WCX (weak cation exchanger, carboxylate functional group) or DEAE (weak anion exchanger, diethylaminoethyl group), or PSA (N-propylethylenediamine groups). Table 1 lists the pKa of Weak Ion exchange beads.

	Beads	pKa
Weak Anion Exchange Magnetic beads	DEAE Magnetic Beads	A Weak Anion Exchange (WAX) Beads, pKa =~11.5
	PSA Magnetic Beads	A Weak Anion Exchange (SAX) Beads, pKa =~10
Weak Cation Exchange Magnetic beads	WCX Magnetic Beads	A Weak Cation Exchange (SCX) Beads, pKa =~5

Table1. pKa of Weak Ion Exchange beads.

- The magnetic bead binding capacities: >60 µg Lysozyme/ mg of Beads for WCX assayed in 25 mM sodium acetate buffer, pH 5.5, or >50 µg BSA / mg of Beads for DEAE, or >60 µg BSA / mg of Beads for PSA determined in 25 mM Tris•HCl, pH 8.0.
- Based on the protein's pI, empirically calculate the appropriate buffer (pH and salt concentration) for purifying and eluting the protein of interest Fig.1). In a buffered solution above the protein's pI, the protein becomes negatively charged (deprotonated) and binds to the positively charged functional groups of an anion exchange resin. To choose the correct buffer for a selected pH, the following is a general rule for selecting a buffer pH:

  Anion exchanger — 0.5–1.5 pH units higher than the protein's pI of interest.

  Cation exchanger — 0.5–1.5 pH units lower than the protein's pI of interest.

Ion-exchanger	Weak anion	Strong anion	Weak cation	Strong cation
Functional groups charge	Positive		Negative	
Biomolecule net charge	Negative		Positive	
pH range	5-9	0-14	5-9	0-14
Running buffer pH	0.5-1 unit higher than buffer pH		0.5-1 unit lower than buffer pH	

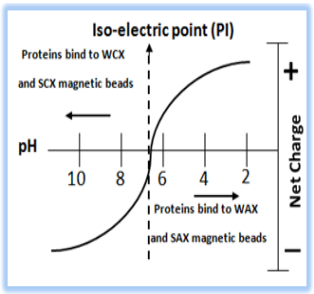

Fig.1

- For the anion beads, ensure that the buffer pH is greater than the protein's isoelectric point and that the sample does not contain anionic detergents. Use a buffer with a pH of 5 to 10 and a salt concentration of ≤ 25 mM to purify the sample. Use a stepwise salt gradient to achieve a final salt concentration of 2 M for elution.
- For the cation beads, ensure that the buffer pH is less than the protein's isoelectric point and that the sample contains no cationic detergents. Use a buffer with a pH range of 3 to 8 and a salt content of ≤ 25 mM to purify the sample. Use a stepwise salt gradient to achieve a final salt concentration of 2 M for elution.
- For successful purification, dilute, dialysis, and gel filtrate the protein sample before mixing with the magnetic beads. Dilute the material with buffer to ≤ 25 mM salt for the best results.

Protocol

a. Equipment

- Magnetic rack (for manual operation)
  Based on sample volume, the user can choose one of the following magnetic racks: BcMag rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag 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™ rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05). For larger scale purification, Ceramic magnets Block for large scale purification (6 in x 4 in x 1 in block ferrite magnet, Applied Magnets, Cat# CERAMIC-B8)
- Corning 430825 cell culture flask for large-scale purification (Cole-Parmer, Cat#EW-01936-22)
- Mini BlotBoy 3D Rocker, fixed speed, small 10" x 7.5" platform w/ flat mat (Benchmark Scientific, Inc. Cat# B3D1008) or compatible

b. Sample

- Binding/Washing buffer: The pH of the Binding Buffer should be at least one pH unit away from the pI of the target protein or peptide.(Note: For best results, the salt should be ≤ 25mM in the sample). If necessary, dilute the sample with purification buffer to minimize its ionic strength. For purifying Basic Proteins (high pI) with Weak Cation Magnetic Beads, for example, Binding/Washing buffer (50 mM Sodium phosphate, pH 7). For purifying Acidic Proteins (low pI) with Weak Anion Magnetic beads, for example, Binding/Washing buffer (25 mM Tris•HCl buffer, pH 8.0).
- Elution Buffer**:** To elute the target protein or peptide from the magnetic beads, the user should optimize elution conditions for individual application by using solutions with increasing salt concentration, i. e., increase stepwise to a final salt concentration of 2.5 M) or changing the pH of the elution buffer. For purifying Basic Proteins (high pI) with Weak Cation Magnetic Beads, for example, Elution buffer (50 mM Sodium phosphate, pH 7.0, 0.1-1.0 M NaCl). For purifying Acidic Proteins (low pI) with Weak Anion Magnetic beads, for example, Elution buffer (25 mM Tris•HCl buffer, pH 8.0 containing 0.5 or 1.0 M NaCl).

A. General Protocol for using the Magnetic beads

a. Weak Ion Exchange magnetic beads preparation

1. Vigorously shake the bottle until the magnetic beads become homogeneous and transfer an appropriate volume of the magnetic beads from the bottle to a new tube or flask.

Note:

- Optimize the number of beads used for each application. Too many beads will cause higher background. Insufficient beads will lead to lower yields.
- Do not allow the beads to sit for more than 3 minutes before dispensing. Resuspend the magnetic beads *every 3 minutes.*

2. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Add ten bead-bed volumes of dH_2O_2 and mix the beads by pipetting or vortex. Again, place the tube on the magnetic rack for 1-3 minutes and remove the supernatant while the tube remains on the rack.
3. Repeat step (2) one more time.
4. Equilibrate the beads by adding ten bead-bed volumes of Binding/Washing buffer and shake it to mix them. Incubate at room temperature with continuous rotation for 2 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. The beads are ready for purification.

b. Purification

1. Add the equilibrated beads (Step a (4) to the sample and incubate on Mini BlotBoy 3D Rocker with continuous rotation for 5-10 minutes.
2. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Add ten bead-bed volumes of Binding/Washing buffer and shake it ten times to wash the beads. Again, place the tube on the magnetic rack for 1-3 minutes and remove the supernatant while the tube remains on the rack.
3. Repeat step (2) six times.

   Note:

   - This step is critical to get high pure protein. It may be necessary to wash the beads more than six times for some proteins to reduce the nonspecific binding.

- Optimize the washing buffer (pH and salt concentration)

Elute protein with an appropriate volume of elution buffer by pipetting up and down 10-15 times or vortex mixer for 5 minutes.

Note: Determine the optimum elution buffers (pH and salt concentration)**,** and it may be necessary to elute the protein 2-3 times.

4. Elute protein with an appropriate volume of elution buffer by pipetting up and down 10-15 times or vortex mixer for 5 minutes.

Note: Determine the optimum elution buffers (pH and salt concentration)**,** and eluting the protein 2-3 times may be necessary.

5. Collect and transfer the supernatant to a new tube.

B. Troubleshooting

Problem	Possible Causes	Suggestions
Low yield	The sample's ionic strength is high.	• The sample should be dialyzed, desalted, or diluted in a salt ≤25 mM purification buffer.
	ample contains interfering detergents.	
The protein failed to elute.	Ionic interaction is too strong.	• Increase the NaCl concentration • Decrease pH of the Elution Buffer. • Using Weak Anion Exchange Magnetic Beads
Poor separation	Carry-over between eluted fractions	• Add more wash steps between each elution step
	Proteins or peptides with similar pI to the target protein	• Optimize NaCl concentration and/or pH of the Elution Buffer

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	

General References

1. Staby A, Jensen RH, Bensch M, Hubbuch J, Dünweber DL, Krarup J, Nielsen J, Lund M, Kidal S, Hansen TB, Jensen IH. Comparison of chromatographic ion-exchange resins VI. Weak anion-exchange resins. J Chromatogr A. 2007 Sep 14;1164(1-2):82-94.
2. Olomukoro AA, Emmons RV, Godage NH, Cudjoe E, Gionfriddo E. Ion exchange solid phase microextraction coupled to liquid chromatography/laminar flow tandem mass spectrometry for the determination of perfluoroalkyl substances in water samples. J Chromatogr A. 2021 Aug 16;1651:462335.
3. Boudesocque L, Lameiras P, Amarouche N, Giraud M, Quattrini F, Garrity JM, Nuzillard JM, Renault JH. Ion-exchange centrifugal partition chromatography: a methodological approach for peptide separation. J Chromatogr A. 2012 May 4;1236:115-22.
4. Noh H, Yohe ST, Vogler EA. Volumetric interpretation of protein adsorption: ion-exchange adsorbent capacity, protein pI, and interaction energetics. Biomaterials. 2008 May;29(13):2033-48.
5. Velayudhan A, Horváth C. Preparative chromatography of proteins analysis of the multivalent ion-exchange formalism. J Chromatogr. 1988 Jun 29;443:13-29.