Weak Cation Exchange (WCX) Magnetic Beads

Introduction

Magnetic Beads possess the capacity to fractionate proteins or nucleic acids through the utilization of beads-adsorbent technology that acts as a chromatographic matrix. The process of ion exchange chromatography is prevalently employed for the separation or purification of a target molecule from raw biological materials. The principle behind this procedure is the divergence in surface charges' accessibility among the molecules, wherein the process utilizes minimal binding and eluting conditions to maintain the biological activity's integrity.

BcMag™ Weak Cation Exchange (WCX) Magnetic Beads are uniform magnetic beads grafted with a high density of a carboxylate functional group (Fig.1) on the surface. The 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 Cation Exchange resins allow the rapid release of very strong ions that may be retained irreversibly on Strong Ion Cation (SCX) beads. Additionally, weak ion exchangers can be effective separation tools when strong ion exchangers fail because the selectivity of weak and strong ion exchangers frequently differ.

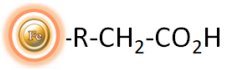

Fig.1 WCX structure

WEAK CATION EXCHANGE (WCX) magnetic beads are used to replace time-consuming, complex, and costly chromatographic procedures such as agarose, cellulose, Sepharose, and Sephadex-based columns or resins. 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 reasonably constant nucleic acid yields.

Magnetic resins have significant advantages over non-magnetic resin technologies. It is due to the numerous benefits of magnetic beads, 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 simple**—WCX Magnetic resins-based format eliminates columns or filters or a laborious repeat of pipetting or centrifugation.
- **Convenient and expandable**— WCX Magnetic format enables high-throughput processing of multiple samples in parallel with many different automated liquid handling systems.
- **Robust**—Magnetic beads 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.

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 before 1D or 2D PAGE
- Phosphopeptide purification before MS analysis

Specificities	
Composition	Magnetic beads grafted with the weak cation-exchange group.
Number of Beads	~ 1.68 x 10^9 beads/mg (1µm beads) ~ 5 x 10^7 beads/mg (5µm beads)
Magnetization	~45 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.0 g/ml
Stability	Most organic solvents
Weak cation exchange Beads	1.0 µm beads: >2.5 mg Lysozyme / ml of Beads
	5 µm beads: >2 mg Lysozyme / ml of Beads
Storage	Store at 4°C upon receipt

Protocol

Note: The following protocol is an example of fractionating a protein or peptide sample with BcMag™ WCX magnetic beads. Users are encouraged to choose alternative binding, washing, or elution buffers to get the best results and determine the optimal working conditions based on the protocol and suggestions described in the troubleshooting section. It is critical to match the amount of the beads to the amount of protein in the starting material in all protein purification experiments. It is not only for financial reasons but also because insufficient WCX resin results in inadequate protein binding in the solution. Too many affinity binding sites will result in the binding of other proteins, making the purification less selective and requiring extra purification steps to achieve pure protein. We recommend performing a titration to optimize the beads used for each application. It is necessary to optimize volumes of elution to avoid unnecessary sample dilution.

Note: Select the appropriate buffer

- Based on the protein's pI, empirically calculate the appropriate buffer (pH and salt concentration) for purifying and eluting the protein of interest Fig.2). 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	

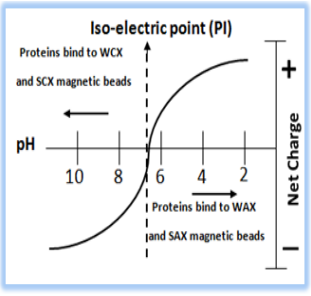

Fig.2

- **Binding/Wash Buffer:** The pKa of the BcMag™ weak cation exchange magnetic beads is about 5.0. The pH of Binding/Wash Buffer should be 7.0 or above and at least one pH unit away from the pI of the target protein or peptide. At pH 7.0 or above, the beads carry a negative charge.

- **Elution Buffer:** To elute the target protein or peptide from the magnetic beads, user should optimize elution condition for individual application by stepwise elution either using solutions with increasing salt concentration (50 mM sodium phosphate pH 7.0, 50 mM NaCl, followed by 50 mM sodium phosphate pH 7.0 100 mM NaCl, then 50 mM sodium phosphate pH 7.0, 200mM NaCl, then 50 mM sodium

phosphate pH 7.0, 400 mM NaCl, then 50 mM sodium phosphate pH 7.0, 800 mM NaCl, and 50 mM sodium phosphate pH 7.0, 1.0 M NaCl), or using 0.5% trifluoroacetic acid (TFA) in water.

A. Equipment

- 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). 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. Buffer

- Precondition Buffer: 50 mM Sodium phosphate, pH 7.
- Binding/Wash Buffer: 50 mM Sodium phosphate, pH 7.0, 20 mM NaCl
- Elution Buffer: 50 mM Sodium phosphate, pH 7.0, 0.1-1.0 M NaCl

General Protocol for using the weak cation exchange magnetic beads.

a. Weak cation 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 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 eluting the protein 2-3 times may be necessary.

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

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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.
	The sample contains interfering detergents.	
The protein failed to elute.	Ionic interaction is too strong.	• Increase the NaCl concentration. • Decrease pH of the Elution Buffer.
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	