BcMag™ C- 4, C-8, Phenyl, diPhenyl Magnetic Beads

Introduction

The BcMag™ beads, including C-4, C-8, Phenyl, or diPhenyl magnetic beads, are characterized by their uniformity and superparamagnetic nature. They contain hydrophobic groups on their surface that facilitate their efficient functioning as a chromatographic matrix. These beads are particularly designed to enable manual or automatic purification, desalination, and concentration of peptides or proteins within the femtomolar to the picomolar range, thereby alleviating the need for repetitive pipetting and centrifugation procedures that can be both time-consuming and arduous.

Hydrophobic interaction chromatography (HIC)

Hydrophobic interaction chromatography (HIC) is a technique for separating macromolecules from one another based on a reversible interaction between the external hydrophobic region of a biological macromolecule and the hydrophobic ligand (such as phenyl, octyl, or butyl) of a HIC medium. The interaction is enhanced by a buffer with a high salt concentration and reduced with a low salt concentration. Therefore, based on salt concentration in a buffer, the protein with less hydrophobicity is eluted first, whereas the protein with more hydrophobicity elutes last. Compared with other chromatography methods, HIC is a more popular method for separating and purifying protein and peptides in analytical and preparatory scale applications since it employs a less denaturing environment.

Our Butyl, Phenyl, and Octyl are hydrophobic interaction chromatography (HIC) magnetic resins with carbon chains of C4, benzene ring, and C8, respectively. They are specially designed for the purification of organic molecules primarily based on their hydrophobicity profiles.

BcMag™ C-4 magnetic beads (Fig.1) are most suitable for larger molecular weight proteins and peptides. Use this less retentive phase to release hydrophobic molecules by weaker organic solvents rapidly.

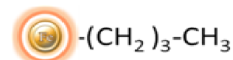

Fig.1 Structure of C-4 magnetic beads

Good matrix for fractionation of hydrophobic molecules which are bound too strongly to C-18 beads to be eluted. BcMag™ C-8 magnetic beads (Fig.2) are most suitable for low to intermediate molecular weight proteins. However, the three types of beads can be used interchangeably in many cases.

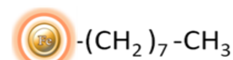

Fig.2 Structure of C-8 magnetic beads

BcMag™ Phenyl Magnetic Beads (Fig.3) are similar in polarity to C8 beads. However, an electron-dense aromatic ring offers some unique selectivity and retention.

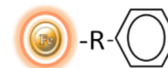

Fig.3 Structure of Phenyl magnetic beads

BcMag™ diPhenyl Magnetic Beads (Fig.4) are used for polypeptides with aromatic side chains, large, hydrophobic proteins, membrane-spanning.
peptides, lipid peptides, and fusion proteins from inclusion bodies.

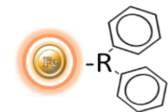

Fig.4 Structure of Phenyl magnetic beads

Hydrophobic magnetic beads

Bioclone hydrophobic magnetic resins are designed as uniform magnetic beads grafted with a high density of hydrophobic ligands on the surface. The hydrophobic magnetic beads are rigid polymeric beads with covalent surface chemistries, allowing easier handling and packing while providing more excellent physical and chemical stability—resulting in a robust production process. The beads replace time-consuming, difficult, and expensive chromatographic techniques such as agarose, cellulose, Sepharose, Sephadex-based columns, or resins. The hydrophobic magnetic beads are manufactured using nanometer-scale superparamagnetic iron oxide as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide. The pure inert silica makes less nonspecific binding. The beads are much smaller (1 and 5 µm diameter) in size and are non-porous, which exhibit larger surface area, less nonspecific binding, and higher resolution than porous supports.

Specificities	
Composition	Magnetic beads grafted with Hydrophobic groups
Number of Beads	~ 1.68×10^9 beads/mg (1µm beads) ~ 5×10^7 beads /mg (5µm beads)
Magnetization	~45 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.0 g/ml
Stability	Most organic solvents
Formulation	Lyophilized Powder
Binding capacity	1 µm beads: >20 µg protein/mg of Beads 5 µm beads: >18 µg protein/mg of Beads
Storage	Store at 4°C upon receipt

Protocol

Note:

- To achieve maximum binding to the hydrophobic magnetic beads, TFA (trifluoroacetic acid) or other ion-pairing agents should be between 0.1%–1.0% at a pH of <4. The solvents should be completely removed if samples contain excess organic solvents such as methanol or acetonitrile (ACN). Samples can be dried in a vacuum evaporator and resuspended in sample buffer (below). To optimize binding, detergents in samples should be diluted with 0.1% TFA till SDS <0.1%, or Triton ® <1%, or Tween® <0.5%.
- To avoid excessive beads drying between steps, the entire procedure should be carried out in a timely manner.
- The amount of beads used in each application should be empirically titrated. The volumes can be scaled up or down accordingly. We recommend using 10 µl (0.5 mg) hydrophobic magnetic beads to bind ~ 10 µg protein and 5µl elution buffer for 0.5 mg beads.
- Users are encouraged to determine the optimal working conditions based on the protocol and suggestions described in the Troubleshooting section to get the best results.

Materials Required

- **Buffers**
  Equilibration buffer: 0.5% TFA (trifluoroacetic acid) in 5% ACN (acetonitrile)
  Sample Binding Buffer: 2% TFA in 5% ACN
  Washing buffer: 0.5 % TFA in 5% ACN
  Elution Buffer: 70% ACN
- 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).

Procedure

A. Magnetic Beads Preparation

1. Weight and suspend 50 mg beads with 1ml of 50% methanol.
2. Transfer 10µl (50 mg/ml) of completely suspended magnetic beads to a microcentrifuge tube.
3. Place the tube onto a magnetic rack for 1-3 minutes until the supernatant is clear.
4. Wash the beads with 1 ml of Equilibration buffer by a magnetic rack.
5. Aspirate and discard the supernatant with a pipette while the tube remains in the rack.

B. Sample Binding

1. Mix sample (~10µg protein/ peptide) with 1/3 volume of Sample Binding Buffer and add to the tube containing the beads.
2. Thoroughly mix beads and sample using a pipette and leave at room temperature for 2 minutes to allow proteins to bind to the beads.

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3. Place the tube onto the magnetic rack for 1-3 minutes (no longer than 3 minutes) until the supernatant is clear. Aspirate and discard the supernatant with a pipette while the tube remains in the rack.
4. Remove the tube from the rack and resuspend the beads with 100µl washing buffer.
5. Place the tube onto the magnetic rack for 1-3 minutes until the supernatant is clear. Aspirate and discard the supernatant with a pipette while the tube remains in the rack.
6. Repeat steps 2 to 4 for four times.

C. Elution

1. Remove the tube from the rack, add 5µl elution buffer, resuspend the beads and incubate for 2 minutes at room temperature.
2. Place the tube on the magnetic rack for 1-3 minutes and transfer the supernatant containing the eluted protein to a new tube. (User should optimize elution conditions for individual proteins by adjusting acetonitrile concentrations, such as 20%, 50%, 80%).
3. For MALDI-MS analysis, mix 1µl of the eluate with 1µl of matrix solution and spot 0.5µl onto a MALADI-MS target plate.

Troubleshooting

Problem	Possible Causes	Suggestions
Poor absorption of proteins/Peptides to beads.	Hydrophobic interaction is not strong enough.	Increase the NaCl concentration (up to 0.2 M) used during adsorption
	Biomolecules are not completely solubilized in the sample buffer.	Use denaturing conditions during adsorption. Add Guanidine HCl to the sample to achieve a final concentration between 1– 6 M.
	The sample's chemical properties do not support hydrophobic interaction with reverse-phase beads.	Choose suitable reverse phase beads for your sample.
Poor elution	Hydrophobic interaction is too strong.	Increase the acetonitrile concentration used during elution. Decrease the NaCl concentration used during adsorption
	Proteins/peptides are not readily soluble in organic solutions.	Decrease the organic solvent concentration used during elution
	Protein-bound too tightly to beads	Choose more suitable reverse phase beads for your sample.
Poor yield	The quantity of protein or peptides of interest in the sample is too low.	• If small sample quantities are used, decrease the amount of beads used and the volume of the elution buffer. An elution volume of 10µl acetonitrile per mg of beads is recommended. • Use a larger amount of starting sample.

Related Products	
C-8 Magnetic Beads	Cyanopropyl Magnetic Beads
C-18 Magnetic Beads	Phenyl Magnetic Beads
C-4 Magnetic Beads	diPhenyl Magnetic Beads