C18 Magnetic Beads

Introduction

BcMag™ C18(Reversed-Phase) magnetic Beads are uniform superparamagnetic resins containing hydrophobic C18alkyl groups on their surface. The beads are specifically designed for quickly purifying, desalting, and concentrating femtomolar to the picomolar scale of peptides or proteins, manually or automatically, without laborious repeat pipetting and centrifugation. BcMag™ C18Magnetic beads are recommended for purification, desalting, and concentration of low molecular weight proteins or peptides. For low to intermediate molecular weight proteins, BcMag™ C-8 magnetic beads are preferred. BcMag™ C-4 magnetic beads are most suitable for larger molecular weight proteins and peptides. However, the three types of beads can be used interchangeably in many cases.

What is C18?

Linear alkylsilane phases include C18, C8, and C4. C18 is octyl-decyl silane with 18 carbons linked to magnetic silica beads. As a result, they have more carbons and a longer carbon chain than C8 (8 carbons) or C4 (4 carbons).

Because of the extra carbons, C18 has a larger surface area across which the mobile phase must traverse. It gives the bound phase, and the elutes greater interaction time. As a result, the sample elutes more slowly and with greater separation.

C8 (also known as octyl), on the other hand, has a shorter retention duration and sharper peaks. Small organic chemicals benefit from these column types, but long-chain fatty acids and complex molecules benefit from C18s.

The beauty and simplicity of a C18 stationary phase are that it provides a direct hydrophobic interaction. The hydrocarbon can bind and hold solutes in the mobile phase as they travel through the beads via a weak hydrophobic (and van der Waal force) interaction. With this information, the task becomes very clear: to retain molecules on C18 resins, the chemical must become as neutral or hydrophobic as possible. Nothing could be done with analytes that are already neutral or have no probability of getting charged. However, we can use a buffer to limit the extent of their charge state for weakly acidic and basic substances. Another chemical property that can be employed here is pKa (and pKb). Weak acids and bases exist in two states in solution: neutral and deprotonated (acids) or protonated (bases) (bases). At a particular pH, the concentrations of these two conjugate forms are identical.

C18 Hydrophobic magnetic beads (Fig.1)

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, 2.5, and 5 µm diameter) in size and are non-porous, which exhibit larger surface area, less nonspecific binding, and higher resolution than porous supports.

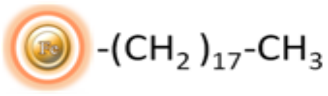

Fig.1 C18 Hydrophobic magnetic beads

Specificities	
Composition	Magnetic beads grafted with C18alkyl groups
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

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Formulation	Lyophilized Powder
Binding capacity	1 µm beads: >20 µg protein/mg of Beads
	5 µm beads: >15 µg protein/mg of Beads
Storage	Store at 4°C upon receipt

Protocol

Note:

- To achieve maximum binding to the BcMag™ C18Magnetic 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) BcMag™ C18Beads for binding of ~ 10 µg protein and 5µl elution buffer for 0.5 mg beads.
- To get the best results, users are encouraged to determine the optimal working conditions based on the protocol and suggestions described in the Troubleshooting section.

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 separator for 1-3 minutes until the supernatant is clear.
4. Wash the beads with 1 ml of Equilibration buffer by a magnetic separator.
5. Aspirate and discard the supernatant with a pipette while the tube remains in the separator.

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.
3. Place the tube onto the magnetic separator 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 separator.
4. Remove the tube from the separator and resuspend the beads with 100µl washing buffer.
5. Place the tube onto the magnetic separator for 1-3 minutes until the supernatant is clear. Aspirate and discard the supernatant with a pipette while the tube remains in the separator.
6. Repeat steps 2 to 4 for four times.

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C. Elution

1. Remove the tube from the separator, add 5µl elution buffer, resuspend the beads and incubate for 2 minutes at room temperature.
2. Place the tube on the magnetic separator 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 from BcMag™ C18Beads	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	
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	