



BcMag™ C-4, C-8 Magnetic Beads

Introduction

BcMag™ C-4, or C-8 (Reversed-Phase) magnetic Beads are ~ 1 µm uniform, silica-based, superpara-magnetic beads containing hydrophobic C4 or C8 alkyl groups on the surface. The beads are specifically designed for quickly purifying, desalting and concentrating femtomolar to picomolar scale of peptides or proteins manually or automatically without the need for laborious repeat pipetting and centrifuging. BcMag™ C-4 magnetic beads are most suitable for larger molecular weight proteins and peptides. BcMag™ C-18 Magnetic beads are recommended for purification, desalting and concentration of low molecular weight proteins or peptides, while BcMag™ C-8 magnetic beads are most suitable for low to intermediate molecular weight proteins. In many cases, however the three types of beads can be used interchangeably.

Product Characteristics

Composition	Silica-coated iron oxide
Bead Size	1µm diameter
Number of Beads	~1.7 x 10 ⁸ beads/mg
Surface Area	~100 m ² /g
Magnetization	~40 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Concentration	Lyophilized Powder
Binding Capacity	>20 µg protein / mg of Beads
Storage	Store at 4°C upon receipt

Note:

- To achieve maximum binding to the BcMag™ C-4 or BcMag™ C-8 Magnetic Beads, TFA (trifluoroacetic acid) or other ion-pairing agents should be between 0.1%–1.0% at a pH of <4. If samples contain excess organic solvents such as methanol or acetonitrile (ACN), the solvents should be completely removed. The sample can be dried in a vacuum evaporator and resuspended in sample buffer 200 mM NaCl, 0.1 TFA. For optimum binding, if samples contain excess detergent, dilute sample with 0.1% TFA till SDS <0.1%, 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 quantity of beads used in each individual application should be empirically titrated. We recommend use of 10 µl (0.5 mg) BcMag™ C-4,C-8 Beads for binding ~ 10 µg protein and 5µl-10µl elution for 0.5 mg beads. The volumes can be scaled up or down accordingly
- In order to get the best results, user should determine the optimal working conditions based on the protocol and suggestions described in the Trouble shooting section

1. Materials Required

- Buffers
Equilibration buffer: 200 mM NaCl, 0.1 % TFA (trifluoroacetic acid)
Sample Binding Buffer: 800 mM NaCl, 0.4 % TFA
Washing buffer: 0.1 % TFA
Elution Buffer: 50% acetonitril in 0.1 % TFA
- Magnetic Separator (for manual operation)
Based on sample volume, user can choose one of the following magnetic Separators: BcMag separator-2 for holding two individual 1.5 ml centrifuge tube, Cat. No. MS-01; BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes, Cat. No. MS-02; BcMag separator-24 for holding twenty-four individual 1.5 ml centrifuge tubes, Cat. No. MS-03; BcMag separator-50 for holding one 50 ml and one 15 ml centrifuge tube, Cat. No. MS-04

2.1 Magnetic Beads Preparation

- Suspend the magnetic beads with 50% Methanol (concentration of 50mg/ml), mix very well by vortex (store at 4°C if not used immediately)
- Transfer 10µl magnetic beads (50 mg/ml) of completely suspended magnetic beads to a microcentrifuge tube.
- Place the tube onto a 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.



5. Remove the tube from the separator and resuspend the beads with 100 μ l Equilibration buffer
6. Repeat steps 2 to 4 for three times.

7. Resuspend the beads with 10 μ l Equilibration buffer.

2.2 Sample Binding

1. Mix sample (~10 μ g protein/ peptide) with 1/3 volume of Sample Binding Buffer and add to the tube containing the washed beads from step 2.1.6.
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 100 μ l 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.

2.3. Elution

1. Remove the tube from the separator, add 5 μ l 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 1 μ l of the eluate with 1 μ l of matrix solution and spot 0.5 μ l onto a MALADI-MS target plate.

Troubleshooting

Problem 1: Poor adsorption of proteins/Peptides to beads

Possible causes:

- Hydrophobic interaction is not strong enough.
Suggestion: Increase the NaCl concentration (up to 0.2 M) used during adsorption
- Biomolecules not completely solubilized in the sample buffer.
Suggestion: Use denaturing conditions during adsorption. Add Guanidine HCl to the sample to achieve a final concentration between 1– 6 M.
- Sample's chemical properties do not support hydrophobic interaction with reverse-phase beads.
Suggestion: Choose suitable reverse phase beads for your sample.

Problem 2: Poor elution from BcMag™ C-4,C-8 Beads

Possible causes:

- Hydrophobic interaction is too strong
Suggestion: Increase the acetonitrile concentration used during elution. Decrease the NaCl concentration used during adsorption
- Proteins/peptides are not readily soluble in organic solutions.
Suggestion: Decrease the organic solvent concentration used during elution.
- Protein bound too tightly to beads.
Suggestion: Choose more suitable reverse phase beads for your sample.

Problem 3: Poor yield

Possible causes:

- The quantity of the proteins or peptide of interest in the sample is too low.
Suggestion:
- If small sample quantities are used, decreasing the amount of beads used and volume of the elution buffer. An elution volume of 10 μ l acetonitrile per mg of beads is recommended
- Use larger amount of starting sample

