

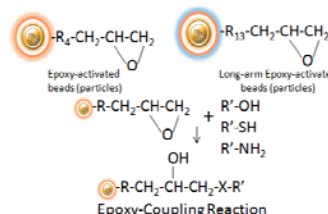
BcMag™ Epoxy-Activated Magnetic Beads

Introduction

BcMag™ Epoxy-Activated Magnetic Beads are pre-activated, uniform, silica-based superpara magnetic beads coated with high density epoxy functional groups on the surface. The beads are used to covalently conjugate amine, sulfhydryl, or hydroxyl group-containing ligands (Fig.1). Coupling of Hydroxyl-, Amine- and Thiol-containing ligands is favored at pH 11-12, pH >9, and pH 7.5-8.5, respectively. Water-insoluble ligands can be conjugated in 50% organic solvent (Dioxane, Dimethylformamide). BcMag™ Epoxy-Activated Magnetic Beads are most suitable for conjugation of large proteins. BcMag™ Long-arm Epoxy-Activated Magnetic Beads are recommended for conjugation of small peptides because the long-arm (21-atom) hydrophilic linker may reduce steric hindrance.

Features and Advantages:

- Pre-activated and ready-to-use
- Covalently couples with high efficiency at pH 9–12, 20°C to 40°C, 16 h–days
- Stable covalent bond with minimal ligand leakage
- Produces reusable immunoaffinity matrices
- Low nonspecific binding
- Immobilize 1-10 mg protein or 0.1-1 mg peptide/ml beads
- Applications: Cell sorting, Immunoprecipitation; Purification for Antibodies, Proteins/Peptides, DNA/RNA



Product Specificities

Composition	Silica-coated iron oxide with high density of epoxy group	
Bead Size	1µm diameter; ~5µm diameter	
Number of Beads	~1.7 x 10 ⁸ beads (1µm beads) /mg; ~5 x 10 ⁷ beads (5µm beads) /mg	
Surface Area	~100 m ² /g	
Stability	Short Term (<1 hour): pH 3-11; Long-Term: pH 4-10 Temperature: 4°C -140°C; Most organic solvents	
Magnetization	~40-45 EMU/g	
Type of Magnetization	Superparamagnetic	
Effective Density	2.5 g/ml	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~250 µmole (1µm beads) / g of Beads
	5µm Magnetic Beads	~195 µmole (5µm beads) / g of Beads
	1µm Long-Arm Magnetic Beads	~200 µmole (1µm beads) / g of Beads
	5µm Long-Arm Magnetic Beads	~172 µmole (1µm beads) / g of Beads
Storage	Store at -20°C, free of moisture upon receipt.	

Note:

- The following protocol is an example for coupling amine-containing ligands to BcMag™ Epoxy-activated magnetic beads.*
- *It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application. If the ligand loading is too low, it may cause nonspecific binding. If the ligand loading is too high, it may cause steric hindrance. 1-10 mg of protein per ml beads or 0.1-1 mg peptide/ml beads is recommended to make affinity matrix. This protocol can be scaled up and down accordingly.*

A. Materials Required

- 1) 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 tubes (Cat. # MS-01); BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag separator-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag separator-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat.# MS-04)
- 2) Coupling Buffer: 0.1 M sodium carbonate buffer or 0.1 M sodium phosphate, pH 8.5-10

Note:

- *Ionic strengths of the coupling buffers are critical to obtain the higher coupling efficiency rate. The coupling buffers should be at minimal ionic strengths, and should not contain any amino (e.g. Tris) thiols, hydroxyls and phenols, But the wash or storage buffers can contain amino thiols, hydroxyls and phenols group.*
- 3) Blocking Buffer: 1 M ethanolamine pH 9.0
 - 4) Wash buffer: PBS, pH 7.4.



B. Protocol

Note: Weight, suspend the magnetic beads with 50% Aceton (Concentration: 30mg/ml), disperse the beads by vigorously vortexing and store at 4°C. *Shake the bottle to completely resuspend the Magnetic Beads before use.*

1. Weight and transfer 30 mg Magnetic beads to a centrifuge tube.
2. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator. Remove the tube from the separator and resuspend the beads with 1 ml coupling buffer by vortex for 30 seconds.
3. Repeat step-2 three times

Note:

- *Once rehydrated, the bead should be used as soon as possible due to stability of functional group. For 1 um beads, some beads may aggregate after beads are suspended in buffer, they can be completely suspended by vigorous vortexing, or very mild sonication for 10-30 seconds*

4. Dissolve or dilute appropriate amount of protein, peptide in 1 ml coupling buffer.

Note:

Coupling efficiencies to epoxy-activated magnetic beads varies from ligand to ligand. The user should empirically optimize the concentration of the ligand. 1-10 mg/ml is recommended for protein conjugation. For small peptides, the concentration of ligand should be at least 200 µmoles ligand per ml.

5. Add the protein solution to the washed beads. Resuspend the magnetic beads and mix very well. Incubate the reaction with continuous rotation.

Note:

- *Amine-containing ligands such as proteins are coupled at 25°C from 15-48 hours. However, if ligand is very sensitive to temperature, it can be conjugated at 4°C for at least 48-72 hours.*
- *Peptides or hydroxyl-containing ligands such as carbohydrates are coupled at from 25-75°C for 4-15 hours.*
- *For thiol-containing ligands, it is recommended to be coupled at from 25-75°C for 4-15 hours.*

6. Wash beads 3 times with 1 ml PBS buffer as described at step 2.
7. Add 0.5-1ml blocking buffer to the beads and incubate at 4°C for at least 4 hours or overnight.
8. Wash beads 4-6 times with 1 ml PBS buffer as described at step 2.
9. Resuspend the beads in PBS buffer with 0.1% azide (w/v) to desired concentration and store at 4°C until use. Do not Freeze.

C. General Affinity Purification Protocol

Note:

Designing a universal protocol for purification of DNA or RNA is relatively straightforward because of nucleic acids relatively uniform biochemical properties. However it is very difficult to design a universal protocol for affinity purification because each protein has a different composition and structure. In order to get the best results, each user must determine the optimal working conditions for individual application.

1. Transfer optimal amount of the beads to a centrifuge tube. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.

Note:

- *It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application based on the amount of the target protein in crude sample. Too many magnetic beads used will cause higher backgrounds, while too little beads used will cause lower yields. Each mg of conjugated magnetic beads normally bind to 1-20 µg target protein.*

2. Remove the tube and resuspend the beads with 5 bed bead volume of PBS buffer by vortex for 30 seconds. Leave the tube at room temperature for 1-3 minutes. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
3. Repeat step 2 two times
4. Add washed beads to crude sample containing target protein and incubate at room temperature or desired temperature for 1-2 hours (Lower temperature require longer incubation time).
5. *Extensively wash the beads with 5 bed bead volumes of PBS buffer or 1M NaCl until the absorbance of elute at 280 nm approaches background level (OD 280 < 0.05).*
6. Elute the target protein by appropriated methods such as low pH (2-4), high pH (10-12), high salt, high temperature, affinity elution or boiling in SDS-PAGE loading buffer.