Epoxy-Activated Magnetic Beads

BcMag™ Epoxy-Activated Magnetic Beads are pre-activated, uniform magnetic beads coated with high-density epoxy functional groups on the surface. The beads can covalently conjugate amine, sulfhydryl, or hydroxyl group-containing ligands (Fig.1). Moreover, the hydrophilic surface ensures beads have low nonspecific adsorption, excellent dispersion, and easy handling in various buffers. The epoxy-activated magnetic beads are most suitable for the conjugation of large proteins. BcMag™ Long-arm Epoxy-Activated Magnetic Beads are recommended to conjugate small peptides because the long-arm hydrophilic linker may reduce steric hindrance.

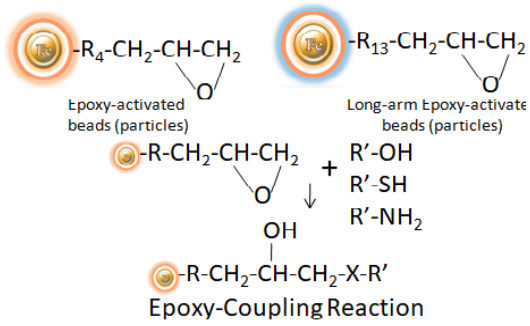

Fig.1 Epoxy-activated coupling

Any ligands such as antibodies, peptides, complete proteins, and functional enzymes can be covalently linked to the bead's surface in a simple overnight reaction. Incubate the ligand you intend to use for affinity purification with these epoxy-activated beads at high pH (8.5-9.5) and 37°C. Coupling can be done in an alternate buffer at pH 7.4 for pH labile ligands.

The unique dry form eliminates the need for acetone solvent storage or removal and disposal. Furthermore, because the dry resin concentrates the sample as it swells, lowering the volume of the starting material and resulting in highly effective ligand immobilization, it is perfect for coupling reactions with dilute materials.

Workflow

BcMag™ Epoxy-Activated Magnetic Beads work perfectly as affinity resin for affinity purification to refine molecules, cells, and parts of cells into purified fractions. After conjugation with ligands, add the beads to a sample containing the target molecules, then mix, incubate, wash and elute the target molecules.

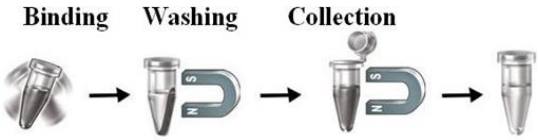

Fig.2

Features and advantages:

- Pre-activated and ready-to-use
- 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

Specification		
Composition	Magnetic beads are grafted with a high density of epoxy groups.	
Number of Beads	~ 1.68 x 10^9 beads/mg (1µm beads) ~ 5 x 10^7 beads /mg (5µm beads)	
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	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~250 µmole / g of Beads
	5µm Magnetic Beads	~195 µmole / g of Beads
	1µm Long-Arm -Magnetic Beads	~200 µmole / g of Beads
	5µm Long-Arm Magnetic Beads	~172 µmole / g of Beads
Storage	Ship at room temperature. Store 4°C upon receipt.	

Protocol

Note:

- *The following protocol is an example of coupling amine-containing ligands to BcMag™ Epoxy-activated magnetic beads.*
- *This protocol can be scaled up as needed. We strongly recommended titration to optimize the number of beads used for each application.*
- *Avoid tris or other buffers containing primary amines or other nucleophiles because these will compete with the intended coupling reaction. But the wash or storage buffers can contain amino.*
- *We recommended performing titration to optimize the quantity of beads used for each application. This protocol can be scaled up and down accordingly.*

Materials required

1. Coupling Buffer: 0.1 M sodium carbonate buffer or 0.1 M sodium phosphate, pH 8.5-10
   Note: *Water-insoluble ligands can be conjugated in 50% organic solvent (dioxane, dimethylformamide). Coupling Hydroxyl-, Amine- and Thiol-containing ligands are preferred at pH 10, pH 9, and pH 7.5-8.5, respectively.*
2. Blocking Buffer: 1 M ethanolamine pH 9.0
3. Wash buffer: PBS, pH 7.4.
4. 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).

A. Magnetic beads preparation

1. Combine 30 mg magnetic beads with 1 ml coupling buffer in a centrifuge tube and mix well.
2. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Remove the tube from the rack and resuspend the beads with 1 ml coupling buffer by vortex for 30 seconds.
3. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant, and the washed beads are ready for coupling.

B. Ligand preparation

1. Dissolve 0.5-10 mg protein, or 200 µmoles peptide**s,** or dilute the appropriate amount of protein and peptide in a 1 ml coupling buffer.
   Note:

- *Coupling efficiencies to epoxy-activated magnetic beads vary from ligand to ligand. The user should empirically optimize the concentration of the ligand. The user should empirically optimize the concentration of the ligand. We recommend 0.5-10 mg/m for protein or 200 µmoles per ml for small peptides.*
- *If samples have already been suspended in other buffers, dilute samples with a 4-fold volume of coupling buffer or desalt or dialyze to buffer-exchange into coupling buffer.*

C. Coupling

1. Add the protein solution to the washed beads. Resuspend the magnetic beads and incubate the reaction with continuous rotation.

   Note:

   - *Immobilize amine-containing ligands such as proteins at 25°C from 15-48 hours. However, if the ligand is very sensitive to temperature, it can be conjugated at 4°C for 48-72 hours.*
   - *Conjugate peptides or hydroxyl-containing ligands such as carbohydrates from 25-75°C for 4-15 hours.*
   - *For thiol-containing ligands, perform conjugation at 25-75°C for 4-15 hours.*
2. Wash beads with 5ml PBS buffer three times.
3. Add -5ml blocking buffer to the beads and incubate at 4°C for at least 4 hours or overnight.
4. Wash beads with 5 ml PBS three times.
5. Resuspend the beads in PBS buffer with 0.01% azide (w/v) to desired concentration and store at 4°C until use. Do not Freeze.

D. General affinity purification Protocol

Note:

- *This protocol is a general affinity purification procedure. Designing a universal protocol for all protein purification is impossible because no two proteins are precisely alike. The user should determine the optimal working conditions for purifying the individual target protein to obtain the best results.*
- *We strongly recommended titration to optimize the number of beads used for each application based on the amount of the target protein in the crude sample. Too many magnetic beads will cause higher backgrounds, while fewer beads will cause lower yields. Each mg of magnetic beads typically binds to 10-20 µg of the target protein.*

1. Transfer the optimal amount of the beads to a centrifuge tube. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
2. Remove the tube and wash the beads with 5-bed volumes of PBS buffer by vortex for 30 seconds. Leave the tube at room temperature for 1-3 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
3. Repeat step 2 two times
4. Add washed beads to the crude sample containing the target protein and incubate at room or desired temperature for 1-2 hours (Lower temperatures require longer incubation time).

   Note: *Strongly recommended to perform a titration to optimize incubation time. More prolonged incubation may cause higher background.*
5. Extensively wash the beads with 5-beads volumes of PBS buffer or 1M NaCl until the absorbance of eluting at 280 nm approaches the background level (OD 280 < 0.05).

   Note: *Adding a higher concentration of salts, nonionic detergent, and reducing agents may reduce the nonspecific background. For example, adding NaCl (up to 1-1.5 M), 0.1-0.5% nonionic detergents such as Triton X 100 or Tween 20, and a reducing reagent such as DTT or TCEP (we usually use 3mM) to the washing buffer.*
6. Elute the target protein by appropriate methods such as low pH (2-4), high pH (10-12), high salt, high temperature, affinity elution, or boiling in an SDS-PAGE sample buffer.

Related Products	
Amine-Terminated Magnetic Beads	Iodoacetyl-Activated Magnetic Beads
DADPA-Activated Magnetic Beads	Peptide conjugation buffer Kit-I
Carboxyl-Terminated Magnetic Beads	Peptide conjugation buffer Kit-II
Epoxy-Activated Magnetic Beads	DVS-Activated Magnetic Beads
Hydrazide-Terminated Magnetic Beads	NHS-Activated Magnetic Beads
Glycoprotein and Antibody Conjugation Kit-I	Hydroxyl-Terminated Magnetic Beads
Glycoprotein and Antibody Conjugation Kit-II	Sulfhydryl-Terminated Magnetic Beads
Aldehyde-Activated Magnetic Beads	Tosyl-Activated Magnetic Beads
Silica-Modified Magnetic Beads	CDI-Activated Magnetic Beads
Alkyne-Activated Magnetic Beads	Thiol-Activated Magnetic Beads
Azide-Activated Magnetic Beads	Cleavable NHS-Activated Magnetic Beads
Cleavable Amine-Terminated Magnetic Beads	Cleavable Azide-Activated Magnetic Beads
Cleavable Carboxyl-Terminated Magnetic Beads	Cleavable Alkyne-Activated Magnetic Beads
Cleavable Epoxy-Activated Magnetic Beads	Cleavable Iodoacetyl-Activated Magnetic Beads
Cleavable Hydrazide-Terminated Magnetic Beads	Cleavable Tosyl-Activated-Magnetic Beads
Cleavable Aldehyde-Activated Magnetic Beads	Streptavidin Magnetic Beads
Boronate Affinity Magnetic Beads	Cleavable Streptavidin Magnetic Beads
Monomer Avidin Magnetic Beads	