Cleavable Epoxy-Activated Magnetic Beads

BcMag™ Cleavable Epoxy-Activated Magnetic Beads are pre-activated, uniform magnetic beads coated with high-density epoxy functional groups on the surface. The beads are suitable for the conjugation of large proteins or small peptides. The beads can covalently conjugate amine, sulfhydryl, or hydroxyl group-containing ligands. Since the active epoxy group is linked with the beads through a built-in cleavable disulfide linker (Fig.1), reducing agents such as DTT or β-mercaptoethanol can cleave and separate the target molecule-ligand complex from the beads. Moreover, the hydrophilic surface ensures low nonspecific adsorption, excellent dispersion, and easy handling in various buffers.

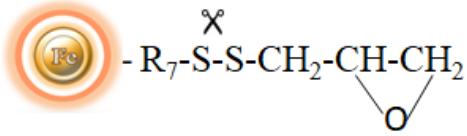

Fig.1

Workflow

The 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)

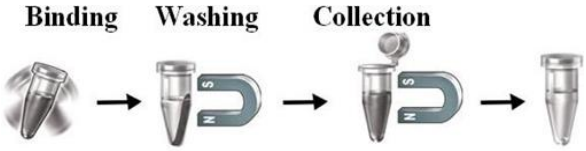

Fig.2

Features and advantages:

- Pre-activated and ready-to-use
- A cleavable built-in disulfide bond allows the ligand-target molecule complex separated from the beads.
- 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: Immunoprecipitation, purification for Antibodies, Proteins/Peptides, DNA/RNA

Specification		
Composition	Magnetic beads are grafted with a high density of cleavable epoxy groups.	
Number of Beads	~ 1.68 x 10^9 beads/mg (1µm beads) ~1.47 x 10^8 beads/mg (2.5µm 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	~255 µmole / g of Beads
	2.5µm Magnetic Beads	~240 µmole / g of Beads
Storage	Ship at room temperature. Store 4°C upon receipt.	

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Protocol

Note:

- *The following protocol is an example for coupling amine-containing ligands to BcMag™ Cleavable 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 reducing agents, tris, or other buffers containing primary amines or other nucleophiles because these will break the disulfide linker or compete with the intended coupling reaction.*
- *We strongly recommended titration to optimize the number of beads used for each application. 1-10 mg of protein per ml beads or 0.1-1 mg peptide/ml beads is recommended to make an affinity matrix. 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:

   - *Water-insoluble ligands can be conjugated in 50% organic solvent (dioxane, dimethylformamide).*
   - *Coupling efficiencies to cleavable epoxy-activated magnetic beads vary from ligand to 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.*
2. 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 based on the following conditions 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.*

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- *Conjugate peptides or hydroxyl-containing ligands such as carbohydrates at 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 5 ml PBS buffer three times.
3. Add 2 ml blocking buffer to the beads and incubate at 4°C for at least 4 hours or overnight.
4. Wash beads with 5 ml PBS four 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. To obtain the best results, the user should determine the optimal working conditions for purifying the individual target protein.*
- *Avoid reducing agents in binding and washing the buffers.*
- *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 used will cause higher backgrounds, while too few beads used 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 5ml 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 temperature or desired temperature for 1-2 hours (Lower temperature 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,* the *addition of NaCl (up to 1-1.5 M) and 0.1-0.5% nonionic detergents such as Triton X100 or Tween20 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 SDS-PAGE sample buffer, or reducing agents.
7. Cleave the disulfide bond.

   Note: *Due to conformational variation from ligand to ligand, the user should determine the optimal working conditions such as reducing agent, pH, and temperature for cleaving the disulfide bond of individual ligands. The following is an example of cleaving conjugated GFP from the beads.*

   1) Incubate the magnetic beads (30mg/ml) in either 140 mM β-mercaptoethanol or 5mM DTT (Dithiothreitol)

      a. 100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 140 mM β-mercaptoethanol for 2 hours to overnight at room temperature or 98°C for 5 minutes.

      b. 100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 5mM DTT for 2 hours to overnight at room temperature or 98°C for 5 minutes.

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	