Cleavable Amine-Terminated Magnetic Beads

BcMag™ Cleavable Amine-Terminated Magnetic Beads are magnetic beads coated with a high density of primary amine functional groups on the surface. The beads can covalently conjugate primary amine or carboxy-containing ligands such as protein and peptides for affinity purification. Since the active amine 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 beads low nonspecific adsorption, excellent dispersion, and easy handling in various buffers. The beads are suitable for the conjugation of large-size proteins or small peptides.

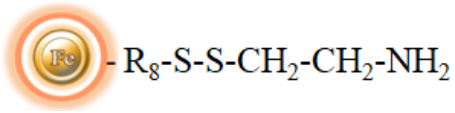

Fig.1

Workflow

The amine 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)

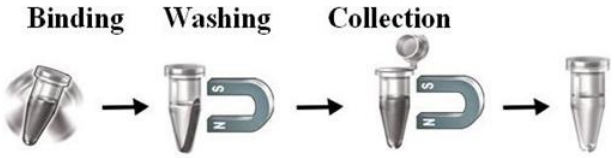

Fig.2

Features and benefits:

- Covalently coupled with high efficiency
- Easy to use.
- Stable covalent bond with low levels of ligand leakage
- Produces reusable immunoaffinity matrix.
- Low nonspecific binding
- Immobilize 1-10 mg protein or 0.1-1 mg peptide/ml beads.
- A cleavable built-in disulfide bond allows the ligand-target molecule complex to separate from the beads.
- Application: Purification for antibody, protein/peptide, DNA/RNA, cell sorting, immunoprecipitation

Specification		
Composition	Magnetic beads coated with a high density of cleavable amine group on the surface	
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	
Concentration	30 mg/ml (1 mM ETDA, pH 7.5)	
Functional Group Density	1µm Magnetic Beads	~250 µmole / g of Beads
	2.5µm Magnetic Beads	~240 µmole / g of Beads
Storage	Store at 4°C upon receipt. Do not freeze	

I. Protocol (Carboxy-containing ligand conjugation)

Note:

1. *The following protocol is an example for coupling carboxy-containing ligands to BcMag™ cleavable Amine-terminated magnetic beads. We strongly recommended performing a titration to optimize the number of beads used for each application. This protocol can be scaled up and down accordingly.*
2. *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. But the wash or storage buffers can contain amino or carboxyl groups.*

Materials required.

- 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).
- Coupling Buffer: 10 mM potassium phosphate, 0.15 M NaCl, pH 5.5 or 0.1 M MES Buffer, 0.15 M NaCl, pH 4.5-5.5.

  Note:

  1. *Conjugate water-insoluble ligands in a coupling buffer containing a final concentration of up to 50% purified dioxane or ethylene glycol. If a mixture of buffer solution and an organic solvent has been used, wash the final product with this mixture of buffer solution.*
  2. *The washing procedure should be followed by a wash with distilled water and then a wash with the buffer in the affinity chromatography* stage.
- EDC [1-ethyl-3 (3-dimethyaminopropyl) carbodiimide], Sigma, Cat# E7750
- Wash/Storage Buffer:10 mM Tris base, 0.15 M NaCl, 0.1% (w/v) BSA, 1mM EDTA, 0.01% sodium azide, pH 7.5.
- Blocking buffer: 1 M Glycine, pH 8.0

Coupling

A. Magnetic Beads Preparation

Note:

- *Shake or vortex the bottle to completely resuspend the magnetic beads before using.*
- *Do not allow the magnetic beads to sit for more than two minutes before dispensing.*

1. Combine 30 mg with 1 ml coupling buffer and mix well by vortex or pipette.
2. Insert the tube into a magnetic rack for 1-3 minutes until the supernatant becomes clear. Aspirate and discard the supernatant with a pipette while the tube remains in the rack.
3. The beads are ready for coupling.

B. Coupling

1. Prepare protein solution (0.5-1mg/ml) with coupling buffer and mix with above-washed beads.
2. Freshly prepare 2% EDC solution with coupling buffer. **Note:** use within 15 minutes of preparing.
3. Add 100 µl of 2% EDC solution to the protein solution and mix well.
4. Incubate at room temperature or t at 4°C overnight with good mixing (end-over-end).

C. Remove uncoupled protein.

1. When the reaction is finished, place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
2. Washing the beads with 5 ml Wash/storage buffer three times.
3. Incubate the beads with 1ml of Blocking buffer at room temperature with good mixing (end-over-end) for 1-2 hours.
4. Washing the beads with 5 ml Wash/storage buffer three times
5. Suspend the beads with the desired Wash/storage buffer volume and store them at 4° C.

II. Protocol (Amine-containing ligand conjugation)

Note:

- *The following protocol is an example of coupling primary amine-containing ligands to BcMag™ Amine-terminated magnetic beads. This protocol can be scaled up and down accordingly. It is strongly recommended that a titration be performed to optimize the number of beads used for each application.*
- *The i*
- *onic strengths of the coupling buffers are critical to obtaining a higher coupling efficiency rate.*
- *The coupling buffers should have minimal ionic strengths and contain no amino (e.g., Tris) or carboxyl groups (e.g., acetate, citrate). But the wash or storage buffers can contain amino or carboxyl groups.*

A. Buffer Preparation

Note:

Prepare buffer solution in a chemical fume hood because Glutaraldehyde or pyridine is volatile and toxic.

- Coupling Buffer: 10 mM pyridine
  Add 800 µl pyridine to 900 ml of dH2O. Adjust to pH 6.0 with HCl. Add dH2O to 1 Liter.
- 5% Glutaraldehyde**:** Add 5.0 ml of 25% glutaraldehyde to 20 ml of Coupling Buffer.
- Reaction Stop buffer: 1M Glycine.
  Dissolve 7.5 g Glycine in 90 ml of dH2O. Adjust to pH 8.0 with 10N NaOH. Adjust the final volume to 100 ml with dH_2O.
- Wash Buffer: 10 mM Tris base, 0.15 M NaCl, 0.1%(w/v) BSA, 1 mM EDTA, 0.01% sodium azide
  Dissolve 1.21g Tris base, 8.7g NaCl, 1.0 g BSA, 0.37g EDTA, sodium salt, 1.0 g sodium azide in 900ml dH2O. Adjust to pH 7.4 with HCl. Adjust the final volume to 1 Liter with dH2O.

B. Bead activation

Note: *Shake the bottle to resuspend the Magnetic Beads entirely before use.*

1. Combine 30 mg with 1 ml coupling buffer and mix well by vortex or pipette.
2. Insert the tube into a magnetic rack for 1-3 minutes until the supernatant becomes clear. Aspirate and discard the supernatant with a pipette while the tube remains in the rack.
3. Resuspend the magnetic beads with 1 ml of 5% Glutaraldehyde and shake vigorously. Incubate at room temperature for 3 hours with good mixing (end-over-end).
4. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
5. Wash beads three times with 1ml coupling buffer to remove unreacted Glutaraldehyde.

C. Coupling

Note: *The supplied concentration cannot reach the*

required 0.5-1 mg/ml for some expensive proteins, such as monoclonal antibodies. The BSA should be added to the protein solution to ensure highly efficient coupling to bring

protein concentration to the required level.

1. Prepare 1 ml of protein solution (0.5-1mg/ml) with coupling buffer and mix with above-activated beads very well by vortex or pipette.
2. Incubate at room temperature or 4°C overnight with good mixing (end-over-end).
3. When the reaction is finished, place the tube into the magnetic rack. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
4. Add 1ml of reaction stop buffer into the tube. Shake vigorously to suspend the beads. Incubate at room temperature for 30 minutes.
5. Washing the beads with 1 ml storage buffer three times.
6. Suspend the beads with the desired volume of Storage buffer and store them at 4° C.

III. 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 the purification of the individual target protein.*
- *Avoid reducing agents in binding and washing 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 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 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 ligands to ligands, 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	