Cleavable Carboxyl-Terminated Magnetic Beads

BcMag™ Cleavable Carboxyl-Terminated Magnetic Beads are uniform, silica-based magnetic beads coated with a high density of carboxylic acid groups on the surface. The high density of carboxylic acid groups on their surface allows efficiently covalently conjugate primary amine-containing ligands via a stable amide bond. Since the active carboxyl 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. The cleavable carboxyl magnetic beads are ideal matrices for conjugating large proteins or small peptides. Moreover, the hydrophilic surface ensures low nonspecific adsorption, excellent dispersion, and easy handling in various buffers.

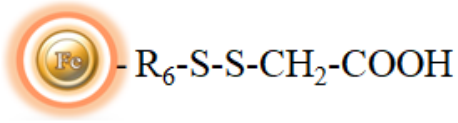

Fig.1

The unique dry form eliminates the need for 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

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)

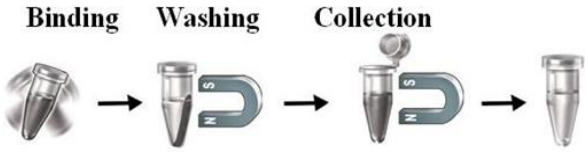

Fig.2

Features and Advantages:

- Easy 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 matrix
- Low nonspecific binding
- Immobilize 1-10 mg protein or 0.1-1 mg peptide/ml beads
- Applications: Purification for antibodies, proteins/peptides, DNA/RNA, cell sorting,immunoprecipitation.

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Specification	
Composition	Magnetic beads grafted with a high density of cleavable carboxyl 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 4-11; 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	~210 µmole / g of Beads
	2.5µm Magnetic Beads	~200 µmole / g of Beads
Storage	Store at 4°C upon receipt. Do not freeze	

Protocol
Note:

1. *The following protocol is an example for coupling protein and peptides to BcMag™Carboxy-terminated magnetic beads. We strongly recommend performing a titration to optimize the number of beads used for each application. The 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.*

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..
- EDC [1-ethyl-3 (3-dimethyaminopropyl) carbodiimide], Sigma, Cat# E7750
- NHS (N-hydroxysuccinimide), Sigma, Cat#56480
- 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

A. Protocol (one-step coupling)

Note:

The one-step coupling is suitable for ligands that do not contain carboxylic acid groups *since carboxylic acid groups may react with the EDC and cause polymerization of the ligand. To compensate for the loss due to polymerization, add excess ligands in the coupling reaction. Since this method is simple and generally yields higher, it is still the preferred coupling method. However, since this method is simple and generally gives higher yields, it is still the preferred coupling method.*

I. Magnetic beads preparation

1. Combine 30 mg with 1 ml coupling buffer in a 1.5 ml centrifuge tube and mix well by vortexing or pipetting.
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.

II. Protein coupling

1. Prepare 1 ml of protein solution (0.5-1mg/ml) with coupling buffer and mix well with the 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% to the protein solution and mix well
4. Incubate for 4 hr or longer at room temperature or overnight at 4°C with good mixing (end-over-end).

III 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 5ml Wash/storage buffer three times
5. Suspend the beads with the desired Wash/storage buffer volume and store them at 4° C.

B. Protocol (two-step coupling)

Note:

Two-step protocol: This protocol is preferred for ligands containing carboxyl groups or you have only limited amounts of ligand.

I. Magnetic beads preparation

1. Combine 30 mg with 1 ml coupling buffer in a 1.5 ml centrifuge tube and mix well by vortexing or pipetting.
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. Freshly prepare 5% EDC and 5% NHS solutions with coupling buffer. **Note:** use within 15 minutes of preparing.
4. Add 500 µl of 5% EDC and 500 µl NHS to the beads and mix well
5. Mix well and incubate at room temperature for 30 minutes with good mixing (end-over-end).
6. After incubation, 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.
7. Washing beads with 5 ml cold coupling buffer three times as described in step 3.
8. The beads are ready for coupling the ligands.

II. Protein coupling

1. Prepare 1 ml of protein solution (0.5-1mg/ml) with coupling buffer and mix with the above-washed beads.
2. Add the protein solution to the pre-activated beads from step A9
3. Incubate for 4 hr or longer at room temperature or overnight at 4°C with good mixing (end-over-end).

III. 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 5ml Wash/storage buffer three times
5. Suspend the beads with the desired Wash/storage buffer volume and store them at 4° C.

C. 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 buffers.*
- *We strongly recommended titration to optimize the concentration 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).

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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) 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 an SDS-PAGE sample buffer. Or elution by 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	