Cleavable NHS-Activated Magnetic Beads

The amine group (-NH_2) is the most common functional target for immobilizing protein molecules: This group can be found at the N-terminus of each polypeptide chain (called the alpha-amine) and in the side chain of lysine (Lys, K) residues (called the epsilon-amine). Because of their positive charge under physiological conditions, primary amines are frequently outward-facing (i.e., on the outside surface) of proteins; hence, they are usually available for conjugation without denaturing the protein structure.

BcMag™ Cleavable NHS-Activated Magnetic Beads are uniform magnetic beads coated with a high density of cleavable NHS (N-hydroxyl succinimide) functional groups on the surface. The magnetic resin utilizes reliable NHS-ester chemistry and does not require the use of dangerous chemicals for immobilization. The beads have less nonspecific binding and no leaking of the coupled ligand. The resin can quickly, efficiently, and covalently conjugate any primary amine-containing ligands by forming stable amide linkages with better than 85% coupling efficiency. Bioclone Cleavable NHS-Activated Magnetic Beads reactions take less than an hour (15–30 min at room temp pH 7–9, 4 hours at 4 °C) and produce more stable linkages substantially. Since the active NHS 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.

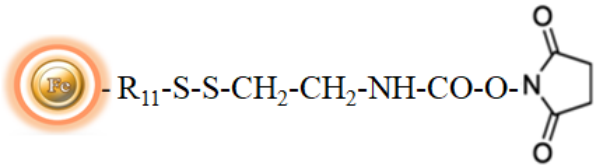

Fig.1 Structure of cleavable magnetic beads

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

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)

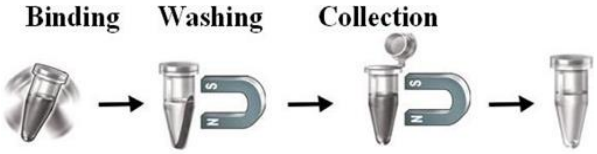

Fig.2

Features and Advantages:

- Pre-activated and ready-to-use
- Cleavable built-in disulfide bond allowing
- the ligand-target molecule complex separated from the beads.
- Easy to use.
- A quick coupling in pH 7.4 at 4°C to 25°C.

- Stable covalent bond with minimal ligand leakage
- Produces reusable immunoaffinity matrices.
- Low nonspecific binding
- Immobilize 15-20 µg protein /mg beads.
- Applications: Affinity purification, immunoprecipitation, purification for Antibodies, Proteins/Peptides, DNA/RNA

Specification		
Composition	Magnetic beads grafted with cleavable NHS 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	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~250 µmole / g of Beads
	2.5µm Magnetic Beads	~235 µmole / g of Beads
Storage	Ship at room temperature. Store at -20°C, free of moisture upon receipt	

Protocol

Note:

- *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. But the wash or storage buffers can contain amino or carboxyl groups.*

A. Materials Required

1. 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).
2. Coupling Buffer: 0.1 M Sodium phosphate, 0.15 M NaCl, pH 7.4
3. Wash Buffer: 0.05 M Tris-HCl, 0.5 M NaCl, pH 8.0.
4. Blocking Buffer: 1 M ethanolamine, pH 9

B. Beads preparation

1. Prepare 3% magnetic beads with 100% acetone (30 mg/ml). Note. Store the unused beads in acetone solution at 4°C. It has been stabling for over a year.
2. Transfer 100 µl (3mg) magnetic beads to a centrifuge tube.
3. 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.
4. Repeat step 3 two times.
5. Remove the supernatant, and the washed beads are ready for coupling.

   Note: *Once rehydrated, use the bead as soon as possible due to the stability of the functional group.*

C. Coupling

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Note: *Coupling efficiencies to NHS-activated magnetic beads vary from ligand to ligand. The user should empirically optimize the concentration of the ligand. For small peptides, the ligand concentration should be at least 200 µmoles per ml. 0.5-10 mg/ml is recommended for protein conjugation.*

1. Dissolve 0.5-10 mg protein in 1 ml coupling buffer.
2. Add 100 µl of the protein solution to the beads. Resuspend the magnetic beads and mix very well by pipetting or vortexing. Incubate the reaction with continuous rotation at room temperature for 4-6 hours or overnight. **Note:** *The user should optimize the incubation time.*
3. 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 wash buffer by vortex for 30 seconds. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
4. Wash beads 3-4 times with 1 ml wash buffer (or 1 M NaCl).
5. Add 0.5-1ml blocking buffer (PBS can also block beads, pH7.4, 0.1% BSA) to the beads and incubate at room temperature for 1 hour or at 4 °*C* overnight.
6. Wash the beads with 1ml of cold Wash buffer 3 times.
7. Resuspend the beads in PBS buffer, pH 7.4, 0.1% BSA, and 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.*
- *Avoid reducing agents in all 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 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), 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 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	

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