

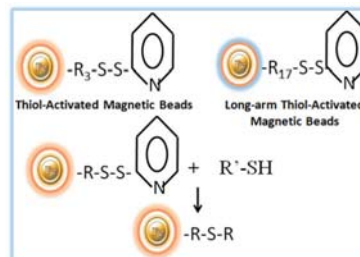
BcMag™ Thiol-Activated Magnetic Beads

Introduction

BcMag™ Thiol-activated Magnetic Beads are uniform, silica-based superparamagnetic beads coated with high density Thiol functional groups (2-pyridyl disulphide) on the surface (Fig.1). The beads are used to reversibly couple thiol-containing ligands. BcMag™ thiol-activated Magnetic Beads are most suitable for conjugation of large proteins. BcMag™ Long-arm thiol-activated Magnetic Beads are recommended for conjugation of small peptides because the long-arm (17-atom) hydrophilic linker may reduce steric hindrance.

Features and Advantages:

- Pre-activated and ready-to-use
- Recommended coupling conditions: pH 4–8, 4°C to 25°C, 3–16 h.
- Specific isolation of cysteine proteins/peptides
- 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



Product Specificities		
Composition	Silica-coated iron oxide magnetic beads grafted with Thiol group on the surface	
Bead Size	~1µm diameter; ~5µm diameter	
Number of Beads	~1.7 x 10 ⁸ beads (1µm beads) /mg; ~5 x 10 ⁷ beads (5µm beads) /mg	
Surface Area	~100 m ² /g	
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	
Effective Density	2.5 g/ml	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~240 µmole (1µm beads) / g of Beads
	5µm Magnetic Beads	~200 µmole (5µm beads) / g of Beads
	1µm Long-Arm-Magnetic Beads	~195 µmole (1µm beads) / g of Beads
	5µm Long-Arm Magnetic Beads	~165 µmole (1µm beads) / g of Beads
Storage	Ship at room temperature. Store at -4°C upon receipt.	

Protocol

Note:

1. *The following protocol is an example for coupling protein and/or peptides to BcMag™ Thiol -Activated magnetic beads. It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application. This protocol can be scaled up and down accordingly.*

A. Materials Required

- **Coupling Buffer** : 0.1 M sodium phosphate, pH 7.0 , 5mM EDTA
- **L-Cysteine•HCl**
- **TCEP (tris(2-carboxyethyl)phosphine)**
- **Washing Buffer**: 1 M NaCl, 0.05% NaN₃
- **Magnetic Separator (for manual operation)**: Based on sample volume, user can choose one of the following magnetic Separators: BcMag separator-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag separator-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag separator-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™ separator-96 for holding a 96 ELISA plate (Cat.# MS-05).



B. Sample Preparation

Note:

- Make sure that protein/peptide to be conjugated has free (reduced) sulfhydryls. To ensure free sulfhydryl groups available, disulfide bonds must be reduced with a reducing agent such as DTT (dithiothreitol), TCEP (tris(2-carboxyethyl)phosphine), or 2-MEA (2-Mercaptoethylamine•HCl) followed by desalting or dialysis to remove the reducing agent.
 - Newly Synthesized peptides may be directly used for coupling, if used immediately after reconstitution
 - For protein, treat protein with 5-10 mM TCEP solution for 30 minutes at room temperature, followed by dialysis or desalting column. For IgG antibody, 2-MEA is recommended due to its Selective reduction of hinge-region disulfide bonds
 - If the sample contains reducing agents with free sulfhydryls (e.g., 2-mercaptoethanol, DTT or TCEP), these agents must be completely removed by dialysis or desalting.
1. Dissolve 1-10mg protein/peptide in 1ml coupling buffer.
 2. If samples have already suspended in other buffer, dilute samples with equal volume of coupling buffer.

C. Magnetic Beads Preparation

Note: Weight, suspend the magnetic beads with 20% Ethanol (Concentration: 30mg/ml), disperse the beads by vigorously vortexing and store at 4°C. Shake the bottle to completely resuspend the Magnetic Beads before use.

1. Transfer 30 mg Magnetic beads to a centrifuge tube. Resuspend the beads by adding 1 ml coupling buffer and mix the beads by vigorous vortexing for 1-2 minutes.
2. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator. Remove the tube from the separator and resuspend the beads with 1 ml coupling buffer by vortex for 30 seconds.
3. Repeat step-2 once.

D. Coupling

1. Add sample from B1 to the washed magnetic beads and incubate for 60 minutes at room temperature with gentle rotation.
2. Washed the magnetic beads with 1ml Coupling buffer for four times as described in B2.
3. Block the excess active groups on the beads by suspending the beads in 1ml Coupling buffer containing 8mg L-Cysteine•HCl and incubate 30-60 minutes at room temperature with gentle rotation.
4. Wash the beads with 1ml Washing buffer four times as described in B3.
5. Resuspend the beads in PBS buffer containing 0.05% sodium azide and store at 4°C.

E. General Affinity Purification Protocol

1. Transfer optimal amount of the beads to a centrifuge tube. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.

Note:

- It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application based on the amount of the target protein in crude sample. Too many magnetic beads used will cause higher backgrounds, while too little beads used will cause lower yields. Each mg of conjugated magnetic beads normally bind to 1-20 µg target protein.
2. Remove the tube and resuspend the beads with 5 bed bead volume of PBS buffer by vortex for 30 seconds. Leave the tube at room temperature for 1-3 minutes. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
 3. Repeat step 2 two times
 4. Add washed beads to crude sample containing target protein and incubate at room temperature or desired temperature for 1-2 hours (Lower temperature require longer incubation time).
 5. Extensively wash the beads with 5 bed bead volumes of PBS buffer or 1M NaCl until the absorbance of elute at 280 nm approaches background level (OD 280 < 0.05).
 6. Elute the target protein by appropriated methods such as low pH (2-4), high pH (10-12), high salt, high temperature, affinity elution or boiling in SDS-PAGE loading buffer.

F. Release the thiol-containing ligand from magnetic beads

1. Resuspend the magnetic beads with 0.1 M DTT (dithiothreitol) or Mercaptoethanol solution and incubate at room temperature for 30 minutes with gentle rotation.
2. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant containing the released ligand to a new centrifuge tube while the tube remains on the separator.
3. Perform buffer change by gel filtration or dialysis to dissolve the ligand into desired buffer.