

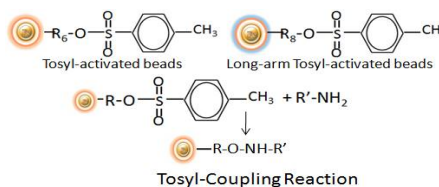
BcMag™ Tosyl Activated Magnetic Beads

INTRODUCTION

BcMag™ Tosyl Activated Magnetic Beads are silica-coated superparamagnetic beads modified with **Tosyl** functional groups on the surface. Both of Tosyl and long-arm tosyl activated magnetic beads can be used to efficiently conjugate primary amine-containing ligands in both aqueous and organic solvents (30% DMF) without introducing any charge (Fig.1). However, the hydrophilic long-arm (18-atoms) tosyl activated magnetic beads are recommended for coupling small molecules.

Features and Advantages:

- Pre-activated and ready-to-use
- Recommended coupling conditions: pH 7.5–9.5 at 37°C with high efficiency
- No charge remain on the surface after coupling
- 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 Characteristics

Composition	Silica-coated iron oxide magnetic beads grafted with Tosyl 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	
Concentration	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~250 µmole / g of Beads
	5µm Magnetic Beads	~210 µmole / g of Beads
	1µm Long-Arm Magnetic Beads	~200 µmole / g of Beads
	5µm Long-Arm Magnetic Beads	~180 µmole / g of Beads
Storage	Ship at room temperature. Store at -20°C, free of moisture upon receipt.	

PROTOCOL

Note:

The following protocol is an example for coupling amine-containing ligands to BcMag™ Tosyl-activated magnetic beads.

- It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application. If the ligand loading is too low, it may cause nonspecific binding. If the ligand loading is too high, it may cause steric hindrance. 1-10 mg of protein per ml beads or 0.1-1 mg peptide/ml beads is recommended to make affinity matrix. This protocol can be scaled up and down accordingly.

A. Materials Required

1. 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)
2. Coupling Buffer: 0.1 M Sodium Carbonate buffer pH 9.5 or 0.1 M sodium phosphate, pH 7.4

Note:

- Ionic strengths of the coupling buffers are critical to obtain the higher coupling efficiency rate. The coupling buffers should be at minimal ionic strengths, and should not contain any amino (e.g. Tris or Glycine). But the wash or storage buffers can contain amino or carboxyl groups.

3. Blocking Buffer: PBS pH 7.4 with 0.5% (w/v) BSA

4. Washing buffer: PBS pH 7.4 with 0.1% (w/v) BSA.

B. Magnetic Beads Preparation



Note: Weight, suspend the magnetic beads with 100% isopropanol (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. Weight and 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.

Note:

- *Once rehydrated, the bead should be used as soon as possible due to stability of functional group.*
- *For 1 um beads, some beads may aggregate after beads are suspended in buffer, they can be completely suspended by vigorous vortexing, or very mild sonication for 10-30 seconds.*

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.

C. Protein coupling

1. Dissolve 1-10mg protein/peptide in 1ml coupling buffer.

Note:

- *Coupling efficiencies to Tosyl-activated magnetic beads varies from ligand to ligand. The user should empirically optimize the concentration of the ligand. 1-10 mg/ml is recommended for protein conjugation. For small peptides, the concentration of ligand should be at least 200 μmoles ligand per ml.*

2. Add the protein solution to the washed beads. Resuspend the magnetic beads and mix very well. Incubate the reaction with continuous rotation at 37°C overnight.

Note:

- *Coupling at 20-25°C requires at least 24-48 hours. While coupling at 4°C, incubation time should be extended to at least 48-72 hours.*

3. Wash beads 3 times with 1 ml washing buffer as described at step B2.
4. Add 0.5-1ml blocking buffer to the beads and incubate at room for 1 hour or at 4 °C overnight.
5. Wash beads 4-6 times with 1 ml PBS buffer as described at step 2.
6. Resuspend the beads in PBS buffer with 0.1% azide (w/v) to desired concentration and store at 4°C until use. Do not freeze

D. 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 sample buffer.