Cleavable Tosyl-Activated Magnetic Beads

BcMag™ Cleavable Tosyl-Activated Magnetic Beads are uniform magnetic beads grafted with tosyl functional groups on the surface.). The tosyl-activated magnetic beads can efficiently conjugate ligands containing primary amine in either aqueous or organic solvents (30% DMF) without introducing any charge. Since the active tosyl group is linked with the beads through a built-in cleavable disulfide linker (Fig.1), after affinity purification, reducing agents such as DTT or β-mercaptoethanol can cleave and separate the target molecule-ligand complex from the beads. The cleavable tosyl magnetic beads are ideal matrices for conjugating large-size proteins or small peptides.

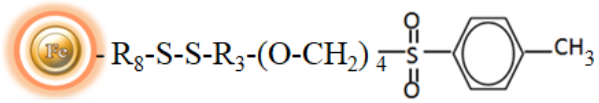

Fig.1. Structure of cleavable Tosyl

The Tosyl-Activated magnetic resins are an ideal choice for covalently attaching antibodies, peptides, complete proteins, and functional enzymes to the surface. The immobilized beads are widely used in the Immunoprecipitation of proteins and protein complexes due to their low background and covalent binding of antibodies to the bead surface.

The Tosyl Activated magnetic resins coupling reaction is carried out at 37°C, and pH ranges from neutral to high. We advocate coupling at pH 8.5-9.5, but coupling with pH labile ligands can be done in an alternate buffer at pH 7.4.

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 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)

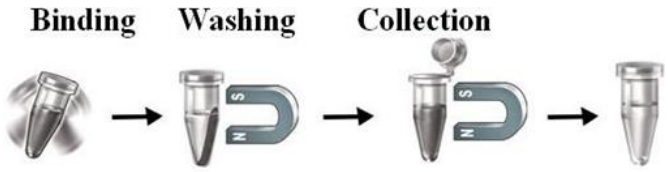

Fig.2

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.
- No charge remains on the surface after coupling.
- 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: Immunoprecipitation; Purification for Antibodies, Proteins/Peptides, DNA/RNA

Specification

Composition	Magnetic beads grafted with cleavable tosyl 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 4-11; Long-Term: pH 4-10 Temperature: 4°C -140°C; Most organic solvents	
Magnetization	~40-45 EMU/g	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~245 µmole / g of Beads
	2.5 µm Magnetic Beads	~230 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 have amino or carboxyl groups.*

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, pH 7.4

   Note:

   - *The coupling buffers should be minimal ionic strengths and should not contain any amino (e.g., Tris or glycine). But the wash or storage buffers can have amino or carboxyl groups.*
   - *Water-insoluble ligands can be conjugated in 30% organic solvent (30% DMF) with a coupling buffer.*
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.

A. Magnetic beads preparation

1. Prepare 3% magnetic beads with 100% isopropanol (30 mg/ml). **Note:** Store the unused beads in acetone solution at 4°C. It is stable 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.*

B. Protein coupling

1. Prepare 100 µl of protein solution (0.5-1mg/ml) or peptide solution (200 µmoles/ml) with coupling buffe.

   Note: *Coupling efficiencies vary from ligand to ligand. The user should empirically optimize the concentration of the ligand*.
2. Add the protein or peptide solution to the washed beads and mix well by vortex or pipette.
3. Incubate the reaction *24-48 hours at* 20-25°C or 48-72 hours at 4°C with continuous rotation.
4. Wash beads three times with 1 ml washing buffer.
5. Add 1ml blocking buffer to the beads and incubate at room for 1 hour or at 4 °*C* overnight.
6. Wash beads 4-6 times with 1 ml PBS buffer.

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7. Resuspend the beads in PBS buffer with 0.01% azide (w/v) to desired concentration and store at 4°C until use. Do not freeze

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. The user should determine the optimal working conditions for purifying the individual target protein to obtain the best results.*
- *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) 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.*

   - 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	