Thiol-activated Magnetic Beads

These chemicals can be used to crosslink proteins and introduce sulfhydryl groups. Pyridyl disulfides form disulfide connections with sulfhydryl groups over a wide pH range (the optimum is pH 4 to 5). A disulfide exchange happens during the reaction between the molecule's -SH group and the reagent's 2-pyridyldithiol group. The disulfide exchange can be conducted at physiological pH. However, the reaction rate is slower.

Applications for pyridyl disulfide immobilization

Because pyridyldithiol compounds generate disulfide bonds with target sulfhydryls, conjugates created with these crosslinkers are cleavable using common disulfide reducing agents, such as dithiothreitol (DTT) or sample buffer for protein electrophoresis (SDS-PAGE). Thus, pyridyldisulfide magnetic beads are useful alternatives to maleimide and haloacetyl reagents when it is necessary to reverse the sulfhydryl-conjugation step later in an experimental procedure (i.e., to exactly recover the original sulfhydryl-containing molecule).

BcMag™ Thiol-activated Magnetic Beads are uniform magnetic beads coated with high-density thiol functional groups (2-pyridyl disulfide) on the surface (Fig.1). The beads can reversibly immobilize thiol-containing ligands under mild conditions. After affinity purification, reducing agents such as DTT or β-mercaptoethanol can cleave and separate the target molecule-ligand complex from the beads. BcMag™ thiol-activated magnetic beads are most suitable for the conjugation of large proteins. BcMag™ long-arm thiol-activated Magnetic Beads are recommended to conjugate small peptides because the long-arm hydrophilic linker may reduce steric hindrance.

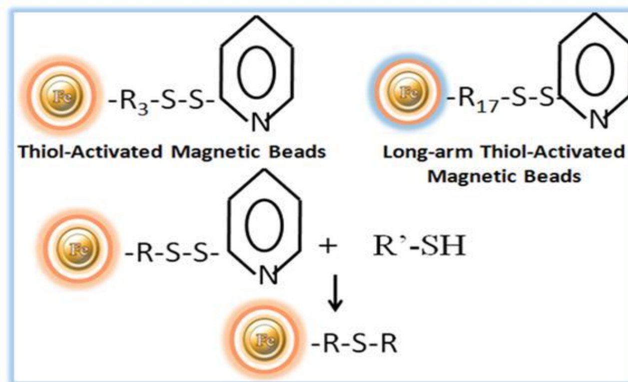

Fig.1 Immobilization of Thiol activated magnetic beads.

Workflow

The magnetic matrix works perfectly as affinity resin for a wide variety of affinity purification to refine thiol group-containing proteins or other molecules from the sample. After washing away unbound material, the thiol-containing substance is eluted by the addition of a reducing agent such as DTT or 2-mercaptoethanol (Fig.2)

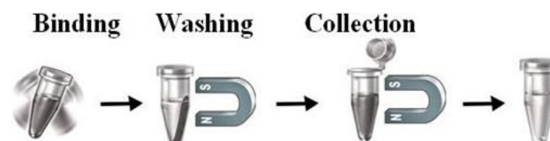

Fig.2

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Features and Advantages

- Pre-activated and ready-to-use
- A cleavable built-in disulfide bond allows the ligand-target molecule.
  Complex separated from the beads.
- Specific isolation of cysteine proteins/peptides
- Stable covalent bond with minimal ligand leakage
- Produces reusable affinity matrix.
- Low nonspecific binding
- Applications: Affinity purification, immunoprecipitation,
  purification of antibodies, proteins/peptides, DNA/RNA

Specification		
Composition	Magnetic beads grafted with Thiol group on the surface.	
Number of Beads	~ 1.68 x 10^9 beads/mg (1µm beads) ~ 5 x 10^7 beads /mg (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	~240 µmole / g of Beads
	5µm Magnetic Beads	~200 µmole / g of Beads
	1µm Long-Arm -Magnetic Beads	~195 µmole / g of Beads
	5µm Long-Arm Magnetic Beads	~165 µmole / g of Beads
Storage	Ship at room temperature. Store at -20°C upon receipt.	

Protocol

Note: *The following protocol is an example for coupling protein and peptides to BcMag™ Thiol-Activated magnetic beads. We strongly recommended titrating the quantity of beads used for each 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 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).

B. Ligand preparation

Note:

- *Make sure that the protein/peptide to be conjugated has free (reduced) sulfhydryl. To ensure free sulfhydryl groups are available, treat the protein/peptide 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 a desalting column. For IgG antibodies, 2-MEA is recommended due to its selective reduction of hinge-region disulfide bonds.*
- *If the sample contains reducing agents with free sulfhydryl (e.g., 2-mercaptoethanol, DTT, or TCEP), these agents must be entirely removed by dialysis or desalting.*

1. Prepare 100 µl of protein solution (0.5-1mg/ml) or peptide solution (200 µmoles/ml) with coupling buffe.
2. If samples have already been suspended in another buffer, dilute samples with an equal volume of coupling buffer.

C. Magnetic Beads Preparation

1. Prepare 3% magnetic beads with Ethanol (30 mg/ml) and mix well.
   Note: *Store the unused beads in acetone solution at 4℃. 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 using the coupling buffer, use the bead as soon as possible due to the stability of the functional group.*

D. Coupling

1. Add the ligand to the washed magnetic beads and incubate at room temperature for 4-6 hours or overnight with continuous rotation.
   Note: *The user should optimize the incubation time*.
2. Wash the magnetic beads with 1ml coupling buffer four times.
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.
5. Resuspend the beads in PBS buffer containing 0.05% sodium azide and store them at 4°C.

E. 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.*

   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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