CDI-Activated Magnetic Beads

CDI-activated support is another alternative for immobilizing amine-containing affinity ligands. When the support reacts with primary amine-containing ligands in an aqueous coupling buffer, the imidazole groups are lost, and carbamate linkages are formed. The coupling process occurs at basic pH (8.5-10); however, it is a slower reaction than reductive amination coupling with proteins. The reaction can also be carried out in an organic solvent to allow the coupling of water-insoluble ligands. Peptides and tiny chemical compounds are particularly well-immobilized by CDI-activated resins.

BcMag™ CDI-Activated Magnetic Beads is a carbonyldiimidazole affinity chromatography resin that has been activated for covalent immobilization of N-nucleophiles and primary amine ligands at pH 9 to 11 in aqueous or organic solvent conditions. It is particularly suitable for conjugating water-insoluble peptides and small organic molecules in organic solvent (Fig.1). BcMag™ CDI-activated magnetic beads are ideal for conjugating a large protein and peptide.

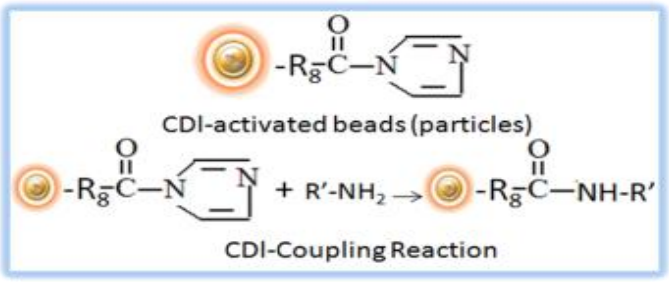

Fig.1 CDI-activated magnetic resin coupling

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

BcMag™ CDI-Activated Magnetic 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)

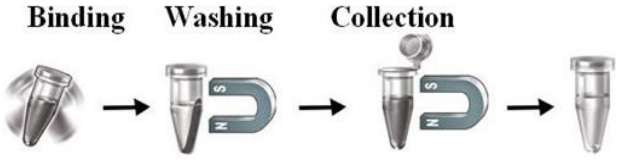

Fig.2

Features and benefits:

- Reliable coupling chemistry—immobilization occurs due to the reaction of N-nucleophiles with the resin's 1,1'-carbonyldiimidazole groups, which results in the formation of stable, uncharged N-alkylcarbamate linkages.
- Simple to use—no secondary reagents are required; wash and equilibrate the resin in alkaline coupling buffer before adding the ligand; the reaction proceeds spontaneously
- Stable activation—the half-life of hydrolysis is much longer than that of hydroxysuccinimide ester activations, making immobilization reactions easier to prepare and control.

Specification		
Composition	Magnetic Bead grafted with CDI 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	~250 µmole / g of beads
	5µm Magnetic Beads	~200 µmole / g of beads
Storage	Ship at room temperature. Store at 4°C upon receipt. Do not freeze	

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 tris or other buffers containing primary amines or other nucleophiles because these will compete with the intended coupling reaction. But the wash or storage buffers can contain amino.*

Materials Required

- 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).
- Coupling/Washing
  1. Soluble ligand coupling buffer: 0.1 M Carbonate buffer, pH 10
  2. Insoluble ligand coupling buffer: Dry acetone, dioxane, or dimethylsulfoxide (DMSO)
- Blocking Buffer: 100 mM Tris, pH 10
- PBS buffer

A. Ligand preparation

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

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

B. Beads preparation

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

C. Coupling

1. Add sample to the washed magnetic beads and incubate at room temperature overnight with continuous rotation.
   Note: *The user should optimize the incubation time*.
2. 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.
3. Repeat step 3 two times.
4. Add 1ml of blocking buffer to the beads and incubate the reaction at room temperature for 4 hours or at 4 °C overnight with continuous rotation.
5. 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 PBS buffer by vortex for 30 seconds.
6. Repeat step 5 for two times.
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

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.*
- *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), 0.1-0.5% nonionic detergents such as Triton X 100 or Tween 20, and a reducing reagent such as DTT or TCEP (we usually use 3mM) 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 an SDS-PAGE sample buffer.

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	