Aldehyde-Activated Magnetic Beads

Introduction

BcMag™ Aldehyde-Activated Magnetic Beads are uniform, silica-based superparamagnetic beads grafted with a high density of aldehyde functional groups on the surface. The bead aldehyde groups react spontaneously with primary amines present at the N-terminus of proteins or in lysine residues to form intermediate Schiff Base complexes (Fig.1). The reaction of reductive amination immobilization starts with the creation of an initial Schiff base between the aldehyde and amine groups, which is then reduced to a secondary amine by the addition of sodium cyanoborohydride ($NaCNBH_3$) to generate stable amine bonds between the Bead and the ligand. At physiological to alkaline circumstances (pH 7.2 to 9) in either aqueous or organic solvents with 20- 30% DMSO or DMF, coupling reaction takes 2 to 6 hours in a one-step process. Coupling efficiency with antibodies and normal proteins is usually better than 85%, resulting in 15 - 20 µg /mg of beads.

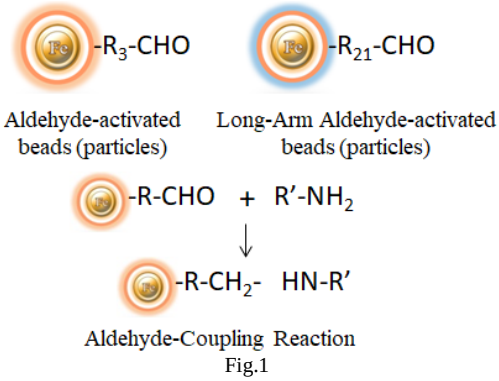

Fig.1

The Aldehyde-Activated resins have higher coupling efficiency than cyanogen bromide (CNBr) activated supports. Furthermore, the aldehyde Chemistry generates an uncharged connection with the amine-containing ligand that is more stable than the CNBr approach. The hydrophilic surface ensures excellent dispersion and easy handling in various buffers. When utilized for affinity purification methods, these features allow better leak-resistant immobilization and lower nonspecific binding. BcMag™ Aldehyde-Activated Magnetic Beads are suitable for conjugating a large protein. At the same time, BcMag™ Long-arm Aldehyde-Activated Magnetic Beads are ideal for conjugating small peptides because the long-arm hydrophilic linker may reduce steric hindrance.

Workflow

BcMag™ Aldehyde-Activate Magnetic Beads work perfectly as solid resin for various affinity purifications to refine molecules, cells, and parts of cells into purified fractions. After conjugation with ligands, add the beads to a solution containing the target molecules, then mix, incubate, wash and elute the target molecules (Fig.2)

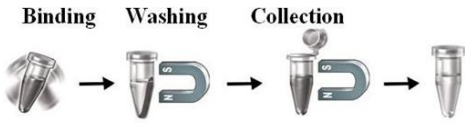

Fig.2 Workflow of Aldehyde-activated resin for affinity purification

Features and benefits

- Quick, Easy, and one-step high-throughput procedure; eliminates columns or filters or a laborious repeat of pipetting or centrifugation.
- Stable covalent bond with minimal ligand leakage

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- High capacity—Immobilize 15 -20 µg antibody/mg beads
- Scalable -easily adjusts for sample size and automation
- Low nonspecific binding
- Reproducible results
- Application: Purification for antibody, protein/peptide, DNA/RNA, cell sorting, immunoprecipitation

Specification		
Composition	Magnetic beads are grafted with a high density of aldehyde groups on the surface.	
Number of Beads	~ 1.68 x 10^9 beads/mg (1µm beads**)** ~ 5x 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	~260 µmole / g of Beads
	5µm **Magnetic** Beads	~200 µmole / g of Beads
	1µm Long-Arm Magnetic Beads	~210 µmole / g of Beads
	5µm Long-Arm Magnetic Beads	~160 µmole / g of Beads
Storage	Ship at room temperature. Store at -20°C 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.

Materials Required

1. Coupling Buffer

   Notes:

   - *The ionic strengths of the coupling buffers are critical to obtaining a high coupling efficiency rate. The coupling buffers should be at minimal ionic strengths and should not contain any amino (e.g. Tris) or other nucleophiles. But the wash or storage buffers can contain amino.*
   - *Prepare buffer solution in a chemical fume hood because sodium cyanoborohydride is very toxic*.

   a. Soluble ligand coupling buffer: 0.1 M sodium phosphate, pH 7.0

   b. Insoluble ligand coupling buffer: 0.1 M sodium phosphate, pH 7, 10-30% acetone, or dioxane, or alcohols, or dimethylformamide (DMF), or dimethylsulfoxide (DMSO) or 6 M Guanidine•HCl or 4 M Urea

2. Blocking Buffer: 1 M Tris•HCl, pH 7.4
3. Washing Buffer: 1 M NaCl
4. Sodium Cyanoborohydride Solution (5 M): NaCNBH3 (MW 62.84) dissolved in 1 M NaOH
5. 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).

A. Magnetic 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.*

B. Protein coupling

1. Dissolve 0.5-10mg protein/peptide in 1ml soluble coupling buffer if soluble. If insoluble, dissolve in 1ml Insoluble coupling buffer. If samples have already been suspended in other buffers, dilute samples with a 4-fold volume of coupling buffer or desalt or dialyze to buffer-exchange into coupling buffer.

   Note:

   - *Coupling efficiencies to Aldehyde-activated magnetic beads vary from ligand to ligand. The user should empirically optimize the concentration of the ligand. We recommend 0.5-10 mg/ml for protein conjugation and at least 200 µmoles ligands per ml for small peptides.*
2. Add 100µl of protein solution and 1µl NaCNBH3 solution (10ul per milliliter of total volume, final concentration 50 mM) to the washed beads in a fume hood. Resuspend the magnetic beads and mix well by pipetting and incubate the reaction at room temperature or 4°C overnight with continuous rotation.
3. Wash beads three times with 1 ml coupling buffer.
4. Add 0.5 blocking buffer and 5µl $NaCNBH_3$ solution ((10ul per milliliter of total volume, final concentration 50 mM) to the beads and incubate in the room for 1 hour or 4 °*C* overnight.
5. Wash beads 4-6 times with 1 ml washing buffer.
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

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. To obtain the best results, each user must determine the optimal working conditions for the purification of the individual target protein.*
- *Strongly recommend performing a 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 will cause higher backgrounds, while too few will cause lower yields. Each mg of conjugated magnetic beads 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 pipetting. 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 temperature or desired temperature for 1-2 hours (Lower temperature require longer incubation time).

   Note: *We strongly recommend performing titration to optimize incubation time. More prolonged incubation may cause higher background.*
5. Extensively wash the beads with 5-bed 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 reagent 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	

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