

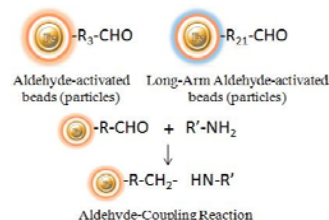
BcMag™ Aldehyde-Activated Magnetic Beads

Introduction

BcMag™ Aldehyde-Activated Magnetic Beads are uniform, silica-based superparamagnetic beads coated with high density of aldehyde functional groups on the surface. The beads are used to covalently conjugate primary amine- containing ligands (Fig. 1). BcMag™ Aldehyde-Activated Magnetic Beads are most suitable for conjugation of larger protein. BcMag™ Long-arm Aldehyde-Activated Magnetic Beads are recommended for conjugation of small peptides because the long-arm (21-atom) hydrophilic linker may reduce steric hindrance.

Features and benefits:

1. coupling condition: pH 4–10, 4–25°C, 2–16 h with high efficiency
2. Water-insoluble ligands can be conjugated in coupling buffer containing final concentration of 10-30% acetone, or dioxane, or alcohols, or dimethylformamide (DMF), or dimethylsulfoxide (DMSO) or In coupling buffer containing final concentration of 6 M Guanidine•HCl or 4 M Urea
3. Stable covalent bond with low levels of ligand leakage
4. Produces reusable immunoaffinity matrices
5. Low nonspecific binding
6. Immobilize 1-10 mg protein or 0.1-1 mg peptide/ml beads
7. Application: Purification for Antibody, Protein/Peptide, DNA/RNA; Cell sorting, Immunoprecipitation
8. Low Cost



Product Specificities		
Composition	Silica-coated iron oxide grafted with high density of aldehyde groups	
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	~260 µmole (1µm beads) / g of Beads
	5µm Magnetic Beads	~200 µmole (5µm beads) / g of Beads
	1µm Long-Arm -Magnetic Beads	~210 µmole (1µm beads) / g of Beads
	5µm Long-Arm Magnetic Beads	~160 µmole (1µm beads) / g of Beads
Storage	Ship at room temperature. Store at -20°C upon receipt.	

Protocol

Note:

1. The following protocol is an example for coupling protein and/or peptides to BcMag™ Aldehyde -Activated magnetic beads. It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application. This protocol can be scaled up and down accordingly.

A. Materials Required

Notes:

1. The ionic strengths of the coupling buffers are critical to obtain the 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.
2. Prepare buffer solution in a chemical fume hood because sodium cyanoborohydride is very toxic.

• Coupling Buffer

1. Soluble ligand coupling buffer: 0.1 M sodium phosphate, pH 7.0
2. 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



- **Blocking Buffer:** 1 M Tris•HCl, 0.05% NaN₃, pH 7.4
- **Washing Buffer:** 1 M NaCl, 0.05% NaN₃
- **Sodium Cyanoborohydride Solution (5 M):** NaCNBH₃ (MW 62.84) dissolved in 1 M NaOH
- **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) ; BcMag™ separator-96 for holding a 96 ELISA plate (Cat.# MS-05).

B. Magnetic Beads Preparation

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 vigorously 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 pipetting.
 3. Repeat step-2 once.

C. 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 suspended in other buffer, dilute samples with 4-fold volume of coupling buffer or desalt or dialyze to buffer-exchange into coupling buffer.

Note:

- *Coupling efficiencies to Aldehyde-activated magnetic beads varies from ligand to ligand. The user should empirically optimize the concentration of the ligand. 0.5-10 mg/ml is recommended for protein conjugation. For small peptides, the concentration of ligand should be at least 200 μmoles ligand per ml.*
2. In a fume hood, add the protein solution and 10μl NaCNBH₃ solution (10ul per milliliter of total volume, final concentration 50 mM) to the beads. Resuspend the magnetic beads and mix very well by pipeting. Incubate the reaction with continuous rotation at room temperature or 4°C overnight.
 3. Wash beads 3 times with 1 ml Coupling buffer as described at step B2.
 4. Add 0.5-1ml blocking buffer and 5-10 μl NaCNBH₃ solution ((10ul per milliliter of total volume, final concentration 50 mM) to the beads and incubate at room for 1 hour or at 4 °C overnight.
 5. Wash beads 4-6 times with 1 ml Washing 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.