

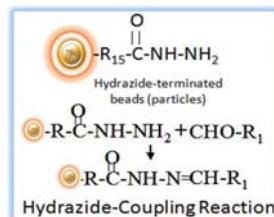
BcMag™ Hydrazide-terminated Magnetic Beads

Introduction

BcMag™ Hydrazide-Terminated Magnetic Beads are uniform, silica-based superparamagnetic beads grafted with high density of hydrazide functional groups on the surface. It is widely used to site-directed Immobilization of aldehyde- or ketone-containing ligands such as antibody, lectin, glycoprotein, and carbohydrates through the formation of stable hydrazone linkages. The beads are especially useful for conjugate polyclonal antibodies since they conjugate only FC portion, the antigen-binding sites are properly oriented and left free.

Features and benefits:

- Covalently couples with high efficiency
- Stable covalent bond with low levels of ligand leakage
- Produces reusable immunoaffinity matrices
- Low nonspecific binding
- Immobilize 1-10 mg protein or 0.1-1 mg peptide/ml beads



Product Specificities		
Composition	Silica-coated iron oxide grafted with Hydrazide group on the surface	
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	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~200 µmole (1µm beads) / g of Beads
	5µm Magnetic Beads	~180 µmole (5µm beads) / g of Beads
Storage	Store at 4°C upon receipt. Do not freeze	

Protocol

Note:

1. The following protocol is an example for coupling glycoprotein to BcMag™ Hydrazide-terminated 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.
2. Tris or other buffers containing primary amines or sugars should be avoided because these will compete with the intended coupling reaction

A. Materials Required

- 1) 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).
- 2) Coupling Buffer: 0.1 M sodium phosphate, pH 7.0 or 0.1 M sodium acetate buffer, pH 5.6
- 3) Oxidizing Agent: Sodium meta-Periodate (NaIO₄) (Sigma, Cat# S1878)
- 4) Sephadex G-25 column
- 5) Washing Buffer: 1M NaCl

B. Protein Coupling

1. Oxidation of Glycoprotein

Note: The reaction is light sensitive and should be performed in the dark.

- 1) Dissolve or dilute 0.5-10 mg glycoprotein in 1 ml coupling buffer. (Note: If the protein is already



suspended in other buffer, perform a buffer exchange by dialysis desalting column.

- 2) Add the protein solution to an amber vial containing 2 mg sodium meta-periodate (final concentration, 10 mM). Swirl gently to dissolve the oxidizing agent.)
- 3) Incubate the sample **in the dark** at room temperature for 30 minutes with gentle rotation.
- 4) Stop the reaction and remove unreacted NaIO_4 by desalting and buffer exchange through Sephadex G-25 column. Equilibrate a 5-ml. Sephadex G-25 column with coupling buffer. Apply the oxidized sample to the column and allow it to enter the gel bed. Apply a 0.5-ml rinse of coupling buffer and allow it also to enter the gel bed. Finally apply 2 ml coupling buffer and collect the eluent.

2. Coupling to BcMag Hydrazide-terminated Magnetic Beads

Note: Weigh, suspend the magnetic beads with 1mM EDTA (Concentration: 30 mg/ml), disperse the beads by vigorously vortexing and store at 4°C. *Shake the bottle to completely resuspend the Magnetic Beads before use.*

- 1) Transfer 0.5 ml magnetic beads (30 mg/ml) of completely suspended magnetic beads to a micro centrifuge tube.
- 2) Insert the tube into a magnetic separator for 1-3 minutes until the supernatant is clear. Aspirate and discard the supernatant with a pipette while the tube remains in the separator. Remove the tube from the separator and resuspend the beads with 1.5 ml Coupling Buffer
- 3) Repeat steps 2 for three times.
- 4) Remove the tube from the separator and resuspend the magnetic beads with 750 μl coupling buffer.
- 5) Mix the magnetic beads with 250 μl oxidized protein solution and incubate at room temperature for at least 6 hours.

Note: *Coupling efficiency depends on the structure and the size of the target glycoprotein. The user should empirically optimize the concentration of the protein. We recommend starting with 100-250 $\mu\text{g/ml}$ for 10 mg of 1 μm beads or 100-150 $\mu\text{g/ml}$ for 10 mg of 5 μm beads.*

- 6) Wash beads with 1 ml washing buffer three times as described in step 2.2.
- 7) Wash beads three times with 1 ml desired storage buffer as described in step 2.2.
- 8) 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. It is impossible to design an universal protocol for all protein purification because no two proteins are exactly alike. In order to obtain the best results, each user must determine the optimal working conditions for purification of individual target protein.

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 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 bead volumes of PBS buffer or 1M NaCl until the absorbance of elute at 280 nm approaches background level ($\text{OD}_{280} < 0.05$).
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.