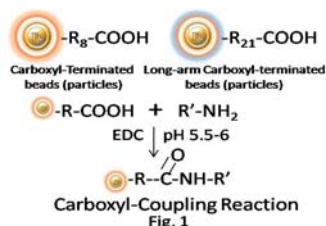


BcMag™ Carboxy-terminated Magnetic Beads

Introduction

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



Product Characteristics

Composition	Silica-coated iron oxide grafted with high density of carboxyl 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	
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
	1µm Long-Arm -Magnetic Beads	~160 µmole (1µm beads) / g of Beads
	5µm Long-Arm Magnetic Beads	~130 µmole (1µm beads) / g of Beads
Storage	Store at 4° upon receipt. Do not freeze	

I. Protocol (one- step coupling)

Note:

- The following protocol is an example for coupling protein and/or peptides to BcMag™ Carboxy-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.
- Ionic strengths of the coupling buffers are critical to obtain the higher coupling efficiency rate.
- The coupling buffers should be at minimal ionic strengths, and should not contain any amino (e.g. Tris) or carboxyl groups (e.g. acetate, citrate). But the wash or storage buffers can contain amino or carboxyl groups.

Materials Required

- 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).
- Coupling Buffer: 10 mM potassium phosphate, 0.15 M NaCl, pH 5.5 (**Note: 0.3-0.5 M NaCl is recommended for Long-arm carboxy-terminated magnetic beads in coupling buffer**)
- Coupling agent: EDC [1-ethyl-3 (3-dimethylaminopropyl) carbodiimide], Sigma, Cat# E7750
- Coupling agent solution: Freshly prepared coupling agent solution by dissolving 57mg EDC in 100 ml ddH₂O. Use immediately after preparation because this solution is unstable.
- Wash/Storage Buffer: 10 mM Tris base, 0.15 M NaCl, 0.1% (w/v) BSA, 1mM EDTA, 0.1% sodium azide, pH 7.5.
- Blocking buffer: 1 M Glycine, pH 8.0

Coupling

A. Magnetic Beads Preparation



Note: Weigh, suspend the magnetic beads with d_2H_2O (Concentration: 20mg/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 10 ml of the beads to a 50ml tube. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
2. Remove the tube and resuspend the beads with 30ml Coupling 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. Resuspend the beads in 10ml of coupling buffer.

B. Coupling of Protein

1. Prepare 10 ml of protein solution (0.5-1mg/ml) with ddH_2O , mix with washed and resuspended beads (Step A 4) and mix very well.
2. Add 4ml of coupling agent (EDC) solution into the tube containing and shake to mix well.
3. Leave reaction for 24 hr at room temperature with gentle rotation. Maintain the pH between 4.5-6.0 with 0.1N HCl during coupling

C. Remove Uncoupled Protein

1. When the reaction is finished, Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
2. Washing the beads with 30 ml Wash/storage buffer three times.
3. Incubate the beads with 5 ml of Blocking buffer at room temperature for 1-2 hours
4. Washing the beads with 30 ml Wash/storage buffer three times
5. Suspend the beads with desired volume of Wash/storage buffer and store at 4° C.

II. Protocol (Two-step coupling)

Note: Two-step protocol: This protocol is preferred for ligands that contain carboxyl groups or you have only limited amounts of ligand available. Normally, one-step protocol provides higher coupling efficiency than two-step protocol.

A. Magnetic Beads Preparation

Note: Weigh, suspend the magnetic beads with d_2H_2O (Concentration: 20mg/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 10 ml of the beads to a 50ml tube. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
2. Remove the tube and resuspend the beads with 30ml Coupling 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. Activate the beads by adding 5 ml EDC solution (add 5mg EDC into 1 ml coupling buffer) and 5ml NHS solution (add 5mg NHS into 1 ml coupling buffer). Mix well.
5. Leave at room temperature for 30 minutes with gentle rotation
6. Washing beads with 30 ml cold coupling buffer two-three times
7. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator. The beads should be used immediately.

B. Coupling of Protein

1. Prepare 10 ml of protein solution (0.5-1mg/ml) with coupling buffer, mix with washed and resuspended beads (Step A 7) and mix very well.
2. Leave reaction for 2-24 hr at room temperature with gentle rotation. Maintain the pH between 4.5-6.0 with 0.1N HCl during coupling

C. Remove Uncoupled Protein

1. When the reaction is finished, Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
2. Wash the beads with 30ml of wash buffer three times.
3. Incubate the beads with 5ml of 1.0 M Glycine, pH 8.0 to block the beads
4. Wash the beads with 30 ml of wash buffer three times
5. Suspend the beads in desired volume of Washing buffer and store at 4° C.

III. 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 protein 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 μ g 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 loading buffer.