

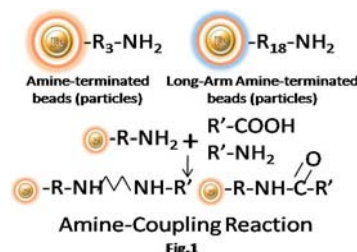
## BcMag™ Amine-terminated Magnetic Beads

### Introduction

BcMag™ Amine-Terminated Magnetic Beads are uniform, silica-based superparamagnetic beads coated with high density of primary amine functional groups on the surface. The beads are used to covalently conjugate primary amine or carboxy- containing ligands (Fig.1). BcMag™ Amine-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.

### 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
- Application: Purification for Antibody, Protein/Peptide, DNA/RNA; Cell sorting, Immunoprecipitation



Product Specificities		
Composition	Silica-coated iron oxide coated with high density of primary amine	
Bead Size	~1µm diameter; ~5µm diameter	
Number of Beads	~1.7 x 10 <sup>8</sup> beads (1µm beads) /mg; ~5 x 10 <sup>7</sup> beads (5µm beads) /mg	
Surface Area	~100 m <sup>2</sup> /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	~250 µmole (1µm beads) / g of Beads
	5µm Magnetic Beads	~200 µmole (5µm beads) / g of Beads
	1µm Long-Arm -Magnetic Beads	~180 µmole (1µm beads) / g of Beads
	5µm Long-Arm Magnetic Beads	~135 µmole (1µm beads) / g of Beads
Storage	Store at 4°C protected upon receipt. Do not freeze	

### Protocol (Carboxy-containing ligand conjugation)

#### Note:

1. The following protocol is an example for coupling carboxy-containing ligands to BcMag™ Amine-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. Ionic strengths of the coupling buffers are critical to obtain the higher coupling efficiency rate.
3. 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.
4. Water-insoluble ligands can be conjugated in coupling buffer containing final concentration of up to 50% purified dioxane or ethylene glycol. If a mixture of buffer solution and an organic solvent has been used, the final product should be washed by this mixture.
5. The washing procedure should be followed by a wash with distilled water and then a wash with the buffer to be used in the affinity chromatography stage.

### 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)
- 2) 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**)
- 3) Coupling agent: EDC [1-ethyl-3 (3-dimethylaminopropyl) carbodiimide], Sigma, Cat# E7750
- 4) Coupling agent solution: Freshly prepared coupling agent solution by dissolving 57mg EDC in 100 ml ddH<sub>2</sub>O. Use immediately after preparation because this solution is unstable.



- 5) Wash Buffer: 10 mM Tris base, 0.5 M NaCl, 0.1% (w/v) BSA, 1mM EDTA, 0.1% sodium azide, pH 7.5.

## Coupling

### A. Magnetic Beads Preparation

**Note:** Weigh and suspend the magnetic beads with 1mM EDTA , pH 7.0 (Concentration: 50mg/ml) and store at 4°C. *Shake the bottle to completely resuspend the Magnetic Beads before use.(Due to small size, it may be necessary to very gently sonicate the beads to completely disperse the bead.*

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 (0.5-2mg/ml) with ddH<sub>2</sub>O, 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 d<sub>2</sub>H<sub>2</sub>O
3. Washing the beads with storage buffer three times.
4. Suspend the beads with desired volume of storage buffer and store at 4° C.

## Protocol (Amine-containing ligand conjugation)

**Note:**

1. *The following protocol is an example for coupling primary amine-containing ligands to BcMag™ Amine-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. *Ionic strengths of the coupling buffers are critical to obtain the higher coupling efficiency rate.*
3. *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.*

### A. Buffer Preparation

**Note:**

*Prepare buffer solution in a chemical fume hood because glutaraldehyde or pyridine is volatile and noxious.*

- **Coupling Buffer: 10 mM pyridine**  
Add 800 µl pyridine to 900 ml of ddH<sub>2</sub>O. Adjust to pH 6.0 with HCl. Add ddH<sub>2</sub>O to 1 Liter
- **5% Glutaraldehyde:**  
Add 5.0 ml of 25% glutaraldehyde to 20 ml of Coupling Buffer.
- **Reaction Stop buffer: 1M Glycine**  
Dissolve 7.5 g Glycine in 90 ml of ddH<sub>2</sub>O. Adjust to pH 8.0 with 10N NaOH. Adjust the final volume to 100 ml with ddH<sub>2</sub>O
- **Wash Buffer: 10 mM Tris base, 0.15 M NaCl, 0.1%(w/v) BSA, 1 mM EDTA, 0.1% sodium azide**  
Dissolve 1.21g Tris base, 8.7g NaCl, 1.0 g BSA, 0.37g EDTA, sodium salt, 1.0 g sodium azide in 900ml ddH<sub>2</sub>O. Adjust to pH 7.4 with HCl. Adjust the final volume to 1 Liter with ddH<sub>2</sub>O.

### B. Bead activation

**Note:** Weigh and suspend the magnetic beads with 1mM EDTA , pH 7.0 (Concentration: 50mg/ml) and store at 4°C. *Shake the bottle to completely resuspend the Magnetic Beads before use.(Due to small size, it may be necessary to very gently sonicate the beads to completely disperse the bead.*

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 magnetic beads by adding 20 ml of 5% Glutaraldehyde and shake vigorously. Leave at room temperature for 3 hr with gentle rotation.
5. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
6. Wash beads three times with 30ml coupling buffer as described to remove unreacted glutaraldehyde.

### C. Coupling of Protein

**Note:**

*For some expensive proteins, such as monoclonal antibodies, the supplied concentration cannot reach the required 2.5-10mg/ml. To ensure high efficient coupling, the BSA should be added to the protein solution to bring protein concentration to the required level.*

1. Prepare protein solution by adding 5-20 mg protein into 10 ml coupling buffer and mix very well.
2. Add the protein solution into the tube containing activated beads (step B6) and Mix well by vigorously shaking. Leave reaction for 24 hr at room temperature with gentle rotation.
3. When the reaction is finished, place the tube into the magnetic separator. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
4. Add 40ml of reaction stop buffer into the tube. Shake vigorously to suspend the beads. Gently shake for 30 min at room temperature.
5. Washing the beads with 30 ml storage buffer three times.
6. Suspend the beads with desired volume of storage buffer and store at 4° C.

### 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 loading buffer.