

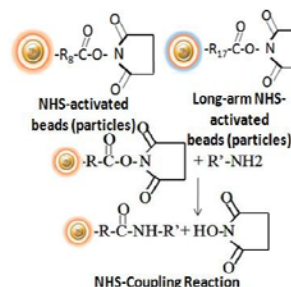
## BcMag™ NHS-Activated Magnetic Beads

### Introduction

**BcMag™ NHS-Activated Magnetic Beads** are uniform, silica-based superparamagnetic beads coated with high density NHS (N-hydroxyl succinimide) functional groups on the surface. The beads are used to specifically conjugate primary Amine-containing ligands (Fig.1), so other active groups in the ligand do not need to be protected prior to coupling. Coupling is fast (15–30 min at room temp pH 6.5–9, 4 hours at 4 °C). BcMag™ NHS-activated Magnetic Beads are most suitable for conjugation of large proteins. BcMag™ Long-arm (17 atome) NHS-activated Magnetic Beads are recommended for conjugation of small peptides because the long-arm (21-atom) hydrophilic linker may reduce steric hindrance.

### Features and Advantages:

- Pre-activated and ready-to-use
- Quick coupling in neutral pH (6.5–9) 4 to 25°C.
- Stable covalent bond with minimal ligand leakage
- Produces reusable immunoaffinity matrices
- Low nonspecific binding
- Immobilize 1-10 mg protein or 0.1-1 mg peptide/ml beads
- Applications: Cell sorting, Immunoprecipitation; Purification for Antibodies, Proteins/Peptides, DNA/RNA



Product Specificities		
Composition	Silica-coated iron oxide magnetic beads grafted with NHS group on the surface	
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	
Formulation	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	~210 µmole (1µm beads) / g of Beads
	5µm Long-Arm Magnetic Beads	~170 µmole (1µm beads) / g of Beads
Storage	Store at -20°C, free of moisture upon receipt	

### Protocol

#### Note:

1. The following protocol is an example for coupling amine-containing ligands to BcMag™ NHS-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.
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) But the wash or storage buffers can contain amino or carboxyl groups.

### 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: 10 mM potassium phosphate, 0.15 M NaCl, pH 5.5 (Note: pH 6.5-9 can be used for coupling. However, the highest coupling efficiency is achieved at pH-5.5.)
3. Wash Buffer: 0.05 M Tris-HCl, 0.5 M NaCl, pH 8.0.
4. Blocking Buffer: 1 M ethanolamine, pH 9



## B. Protein Coupling

1. Weight and transfer 30 mg Magnetic beads to a centrifuge tube.
2. Dissolve 0.5-1 mg protein/peptide in 1ml coupling buffer.

**Note:**

- *Coupling efficiencies to NHS-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  $\mu$ moles ligand per ml.*
3. Add the protein solution to the beads. Resuspend the magnetic beads and mix very well by pipetting or vortexing. Incubate the reaction with continuous rotation at room temperature for 4-6 hours or overnight.
  4. 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 wash buffer by vortex for 30 seconds. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
  5. Wash beads 3-4 times with 1 ml wash buffer (or 1 M NaCl) as described at step 4.
  6. Add 0.5-1ml blocking buffer (Beads can also be blocked by PBS, pH7.4, 0.1% BSA) to the beads and incubate at room temperature for 1 hour or at 4 °C overnight.
  7. Wash the beads with 1ml of cold Wash buffer 3 times as described at step 4.
  8. Resuspend the beads in PBS buffer, pH 7.4, 0.1% BSA and 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 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  $\mu$ g 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 , pH 7.4, 0.5 M NaCl (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.