NHS-Activated Magnetic Beads

The amine group (-NH_2) is the most common functional target for immobilizing protein molecules: This group can be found at the N-terminus of each polypeptide chain (called the alpha-amine) and in the side chain of lysine (Lys, K) residues (called the epsilon-amine). Because of their positive charge under physiological conditions, primary amines are frequently outward facing (i.e., on the outside surface) of proteins; hence, they are usually available for conjugation without denaturing the protein structure.

BcMag™ NHS-Activated Magnetic Beads are uniform, magnetic beads coated with high-density NHS (N-hydroxyl succinimide) functional groups on the surface. The magnetic resin utilizes reliable NHS-ester chemistry and does not require the use of dangerous chemicals for immobilization. The beads have less nonspecific binding and no leaking of the coupled ligand. The resin can quickly, efficiently, and covalently conjugate any primary amine-containing ligands by forming stable amide linkages (Fig.1) with better than 85% coupling efficiency. Bioclone NHS-Activated beads reactions take less than an hour (15–30 min at room temp pH 7–9, 4 hours at 4 ℃) and produce substantially more stable linkages. BcMag™ Long-arm NHS-activated Magnetic Beads are recommended to immobilize small peptides because the long-arm hydrophilic linker may reduce steric hindrance. The magnetic resin can be employed in various affinity purification methods.

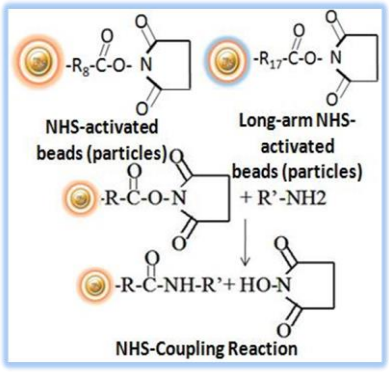

Fig.1 NHS-activated support coupling reaction

The unique dry form eliminates the need for acetone solvent storage or removal and disposal. Furthermore, because the dry resin concentrates the sample as it swells, lowering the volume of the starting material and resulting in highly effective ligand immobilization, it is perfect for coupling reactions with dilute materials.

Workflow

BcMag™ NHS-Activated Magnetic Beads work perfectly as solid support for a wide variety of affinity purification to refine molecules, cells, and parts of cells into purified fractions. After conjugation with ligands, add the beads to a solution containing the target molecules, then mix, incubate, wash and elute the target molecules (Fig.2)

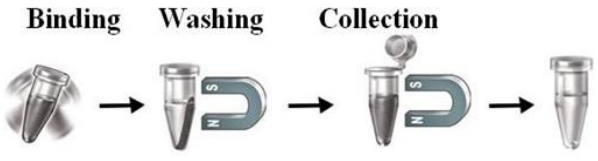

Fig.2

Features and Advantages:

- Pre-activated and ready-to-use
- Easy to use.

Phone: +1-858-909-0079 **Bioclone Inc**. Fax: +1-858-909-0057

- A quick coupling in pH 7.4 at 4°C to 25°C.
- Stable covalent bond with minimal ligand leakage
- Produces reusable immunoaffinity matrices.
- Low nonspecific binding
- Immobilize 15-20 µg protein /mg beads.
- Applications: Cell sorting, Immunoprecipitation; Purification for Antibodies, Proteins/Peptides, DNA/RNA

Specification		
Composition	Magnetic beads grafted with NHS group on the surface.	
Number of Beads	~ 1.68 x 10^9 beads/mg (1µm beads**)** ~ 5 x 10^7 beads /mg (5µm beads)	
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	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~250 µmole / g of Beads
	5µm Magnetic Beads	~200 µmole / g of Beads
	1µm Long-Arm -Magnetic Beads	~210 µmole / g of Beads
	5µm Long-Arm Magnetic Beads	~170 µmole / g of Beads
Storage	Store at -20°C, free of moisture upon receipt	

Protocol

Note:

- This protocol can be scaled up as needed. We strongly recommended titration to optimize the number of beads used for each application.
- Avoid tris or other buffers containing primary amines because these will compete with the intended coupling reaction.

A. Materials Required

1. Magnetic rack (for manual operation): Based on sample volume, the user can choose one of the following magnetic Racks: BcMag rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag rack-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™ rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05).
2. Coupling Buffer: 0.1 M Sodium phosphate, 0.15 M NaCl, pH 7.4
3. Wash Buffer: 0.05 M Tris-HCl, 0.5 M NaCl, pH 8.0.
4. Blocking Buffer: 1 M ethanolamine, pH 9

B. Beads preparation

1. Prepare 3% magnetic beads with 100% acetone (30 mg/ml). Note. Store the unused beads in acetone solution at 4°C. It is stable for over a year.
2. Transfer 100 µl (3mg) magnetic beads to a centrifuge tube.
3. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Remove the tube from the rack and resuspend the beads with 1 ml coupling buffer by vortex for 30 seconds.
4. Repeat step 3 two times.
5. Remove the supernatant, and the washed beads are ready for coupling.

   Note: *Once rehydrated, use the bead as soon as possible due to the stability of the functional group.*

C. Coupling

Note: Coupling efficiencies to NHS-activated magnetic beads vary from ligand to ligand. The user should empirically optimize the concentration of the ligand. The ligand concentration should be at least 200 µmoles per ml for small peptides. 0.5-10 mg/ml is recommended for protein conjugation.

1. Prepare 100 µl of protein solution (0.5-1mg/ml) or peptide solution (200 µmoles/ml) with coupling buffer and mix with the above-washed beads. If samples have already been suspended in another buffer, dilute samples with an equal volume of coupling buffer.
2. Incubate the reaction with continuous rotation at room temperature for 4-6 hours or overnight. N**ote:** *The user should optimize the incubation time.*
3. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Remove the tube from the rack and resuspend the beads with 1 ml wash buffer by vortex for 30 seconds. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
4. Wash beads 3-4 times with 1 ml wash buffer (or 1 M NaCl).
5. Add 0.5-1ml blocking buffer (PBS can also block beads, pH7.4, 0.1% BSA) to the beads and incubate at room temperature for 1 hour or at 4 *°C* overnight.
6. Wash the beads with 1ml of cold Wash buffer 3 times.
   Resuspend the beads in PBS buffer, pH 7.4, 0.1% BSA, and 0.01% azide (w/v) to desired concentration and store at 4°C until use. Do not freeze.

D. General affinity purification Protocol

Note:

- *This protocol is a general affinity purification procedure. Designing a universal protocol for all protein purification is impossible because no two proteins are precisely alike. To obtain the best results, each user must determine the optimal working conditions for the purification of the individual target protein.*
- *We strongly recommended titration to optimize the number of beads used for each application based on the amount of the target protein in the crude sample. Too many magnetic beads used will cause higher backgrounds, while too few beads used will cause lower yields. Each mg of magnetic beads typically binds to 10-20 µg of the target protein.*

1. Transfer the optimal amount of the beads to a centrifuge tube. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
2. Remove the tube and wash the beads with 5-bed volumes of PBS buffer by vortex for 30 seconds. Leave the tube at room temperature for 1-3 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
3. Repeat step 2 two times.
4. Add washed beads to the crude sample containing the target protein and incubate at room or desired temperature for 1-2 hours (Lower temperatures require longer incubation time).
   Note: Strongly recommended to perform a titration to optimize incubation time. More prolonged incubation may cause higher background.
5. Extensively wash the beads with 5-beads volumes of PBS buffer or 1M NaCl until the absorbance of eluting at 280 nm approaches the background level (OD 280 < 0.05).
   Note: *Adding a higher concentration of salts, nonionic detergent, and reducing agents may reduce the nonspecific background. For example, adding NaCl (up to 1-1.5 M), 0.1-0.5% nonionic detergents such as Triton X 100 or Tween 20, and a reducing reagent such as DTT or TCEP (we usually use 3mM) to the washing buffer*.
6. Elute the target protein by appropriate methods such as low pH (2-4), high pH (10-12), high salt, high temperature, affinity elution, or boiling in an SDS-PAGE sample buffer.

Related Products	
Amine-Terminated Magnetic Beads	Iodoacetyl-Activated Magnetic Beads
DADPA-Activated Magnetic Beads	Peptide conjugation buffer Kit-I
Carboxyl-Terminated Magnetic Beads	Peptide conjugation buffer Kit-II
Epoxy-Activated Magnetic Beads	DVS-Activated Magnetic Beads
Hydrazide-Terminated Magnetic Beads	NHS-Activated Magnetic Beads
Glycoprotein and Antibody Conjugation Kit-I	Hydroxyl-Terminated Magnetic Beads
Glycoprotein and Antibody Conjugation Kit-II	Sulfhydryl-Terminated Magnetic Beads
Aldehyde-Activated Magnetic Beads	Tosyl-Activated Magnetic Beads
Silica-Modified Magnetic Beads	CDI-Activated Magnetic Beads
Alkyne-Activated Magnetic Beads	Thiol-Activated Magnetic Beads
Azide-Activated Magnetic Beads	Cleavable NHS-Activated Magnetic Beads
Cleavable Amine-Terminated Magnetic Beads	Cleavable Azide-Activated Magnetic Beads
Cleavable Carboxyl-Terminated Magnetic Beads	Cleavable Alkyne-Activated Magnetic Beads
Cleavable Epoxy-Activated Magnetic Beads	Cleavable Iodoacetyl-Activated Magnetic Beads
Cleavable Hydrazide-Terminated Magnetic Beads	Cleavable Tosyl-Activated-Magnetic Beads
Cleavable Aldehyde-Activated Magnetic Beads	Streptavidin Magnetic Beads
Boronate Affinity Magnetic Beads	Cleavable Streptavidin Magnetic Beads
Monomer Avidin Magnetic Beads	