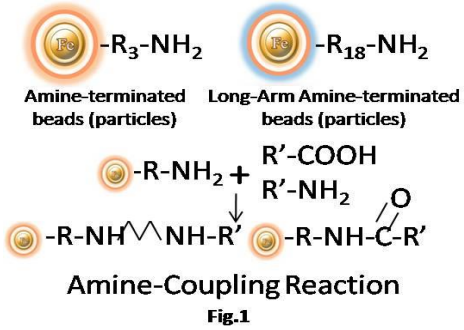
Amine-terminated Magnetic Beads

BcMag™ Amine-terminated Magnetic Beads are magnetic beads coated with a high density of primary amine functional groups on the surface. The beads can covalently conjugate primary amine or carboxy-containing ligands such as protein and peptides for affinity purification (Fig.1). The hydrophilic surface ensures low nonspecific adsorption beads, excellent dispersion, and easy handling in various buffers. BcMag™ Amine-terminated Magnetic Beads are most suitable for conjugating a larger protein. BcMag™ Long-arm amine-terminated Magnetic Beads are recommended to conjugate small peptides because the long-arm (21-atom) hydrophilic linker may reduce steric hindrance.



Amine beads coupling reaction

The carboxyl group is found in a wide range of biological substances. Peptides and proteins contain carboxyls (-COOH) at the C-terminus of each polypeptide chain as well as aspartic acid (Asp, D) and glutamic acid side chains (Glu, E). Carboxyls, like primary amines, are typically found on the surface of protein structures.

Carboxylic acids can be employed to immobilize biological molecules by applying a carbodiimide-mediated process. Although no activated support has a reactive group that is spontaneously reactive with carboxylates, chromatographic supports containing amines (or hydrazides) can be utilized to create amide bonds with carboxylates activated using the water-soluble carbodiimide crosslinker EDC.

Workflow

BcMag™ Amine-Terminated magnetic beads work perfectly as solid resin for various affinity purifications 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)

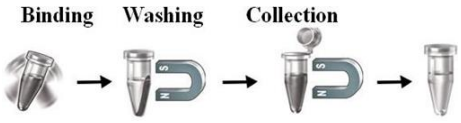


Fig.2

Features and benefits:

- Covalently coupled with high efficiency
- Easy to use.
- 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

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Specification		
Composition	Magnetic beads coated with a high density of primary amine	
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	
Concentration	30 mg/ml (1 mM ETDA, pH 7.0)	
Functional Group Density	1µm Magnetic Beads	~250 µmole / g of Beads
	5µm Magnetic Beads	~200 µmole / g of Beads
	1µm Long-Arm -Magnetic Beads	~180 µmole / g of Beads
	5µm Long-Arm Magnetic Beads	~135 µmole / g of Beads
Storage	Store at 4°C upon receipt. Do not freeze	

I. Protocol (Carboxy-containing ligand conjugation)

Note:

- *The following protocol is an example for coupling carboxy-containing ligands to BcMag™ Amine-terminated magnetic beads. We strongly recommended performing a titration to optimize the number of beads used for each application. This protocol can be scaled up and down accordingly.*
- *The ionic strengths of the coupling buffers are critical to obtaining a higher coupling efficiency rate.*
- *The coupling buffers should have minimal ionic strengths and contain no amino (e.g., Tris) or carboxyl groups (e.g., acetate, citrate). But the wash or storage buffers can contain amino or carboxyl groups.*
- *Conjugate water-insoluble ligands in a coupling buffer containing a final concentration of up to 50% purified dioxane or ethylene glycol. If a mixture of buffer solution and an organic solvent has been used, wash the final product with this mixture of buffer solution.*
- *The washing procedure should be followed by a wash with distilled water and then a wash with the buffer in the affinity chromatography* stage.

Materials Required

- 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).
- Coupling Buffer: 10 mM potassium phosphate, 0.15 M NaCl, pH 5.5 or 0.1 M MES Buffer, 0.15 M NaCl, pH 4.5-5.5. ***Note:*** *For Long-arm carboxy-terminated magnetic beads, adjust NaCl concentration to 0.3-0.5 M (Final concentration) in coupling buffer.*
- EDC [1-ethyl-3 (3-dimethyaminopropyl) carbodiimide], Sigma, Cat# E7750
- Wash/Storage Buffer:10 mM Tris base, 0.15 M NaCl, 0.1% (w/v) BSA, 1mM EDTA, 0.01% sodium azide, pH 7.5.
- Blocking buffer: 1 M Glycine, pH 8.0

Coupling

A. Magnetic Beads Preparation

Note:

- *Shake or vortex the bottle to completely resuspend the magnetic beads before using.*
- *Do not allow the magnetic beads to sit for more than two minutes before dispensing.*

1. Combine 30 mg with 1 ml coupling buffer and mix well by vortex or pipette.
2. Insert the tube into a magnetic rack for 1-3 minutes until the supernatant becomes clear. Aspirate and discard the supernatant with a pipette while the tube remains in the rack.
3. The beads are ready for coupling.

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B. Coupling

1. Prepare protein solution (0.5-1mg/ml) with coupling buffer and mix with above-washed beads.
2. Freshly prepare 2% EDC solution with coupling buffer. **Note:** use within 15 minutes of preparing.
3. Add 100 µl of 2% EDC solution to the protein solution and mix well.
4. Incubate at room temperature or t at 4°C overnight with good mixing (end-over-end).

C. Remove uncoupled protein.

1. When the reaction is finished, place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
2. Washing the beads with 5 ml Wash/storage buffer three times.
3. Incubate the beads with 1ml of Blocking buffer at room temperature with good mixing (end-over-end) for 1-2 hours.
4. Washing the beads with 5 ml Wash/storage buffer three times
5. Suspend the beads with the desired Wash/storage buffer volume and store them at 4° C.

II. Protocol (Amine-containing ligand conjugation)

Note:

- *The following protocol is an example of coupling primary amine-containing ligands to BcMag™ Amine-terminated magnetic beads. This protocol can be scaled up and down accordingly. It is strongly recommended that a titration be performed to optimize the quantity of beads used for each application.*
- *The i*
- *onic strengths of the coupling buffers are critical to obtaining a higher coupling efficiency rate.*
- *The coupling buffers should have minimal ionic strengths and contain no 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 toxic.

- Coupling Buffer: 10 mM pyridine
  Add 800 µl pyridine to 900 ml of ddH_2O. Adjust to pH 6.0 with HCl. Add dH_2O 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_2O. Adjust to pH 8.0 with 10N NaOH. Adjust the final volume to 100 ml with dH_2O.
- Wash Buffer: 10 mM Tris base, 0.15 M NaCl, 0.1%(w/v) BSA, 1 mM EDTA, 0.01% 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_2O. Adjust to pH 7.4 with HCl. Adjust the final volume to 1 Liter with ddH_2O.

B. Bead activation

Note: *Shake the bottle to resuspend the Magnetic Beads entirely before use.*

1. Combine 30 mg with 1 ml coupling buffer and mix well by vortex or pipette.
2. Insert the tube into a magnetic rack for 1-3 minutes until the supernatant becomes clear. Aspirate and discard the supernatant with a pipette while the tube remains in the rack.
3. Resuspend the magnetic beads with 1 ml of 5% Glutaraldehyde and shake vigorously. Incubate at room temperature for 3 hours with good mixing (end-over-end).
4. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
5. Wash beads three times with 1ml coupling buffer to remove unreacted Glutaraldehyde.

C. Coupling

Note:

For some expensive proteins, such as monoclonal antibodies, the supplied concentration cannot reach the required 0.5-1 mg/ml. The BSA should be added to the protein solution to ensure highly efficient coupling to bring protein concentration to the required level.

1. Prepare 1 ml of protein solution (0.5-1mg/ml) with coupling buffer and mix with above-terminated beads very well by vortex or pipette.
2. Incubate at room temperature or t at 4°C overnight with good mixing (end-over-end).
3. When the reaction is finished, place the tube into the magnetic rack. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
4. Add 1ml of reaction stop buffer into the tube. Shake vigorously to suspend the beads. Incubate at room temperature for 30 minutes.
5. Washing the beads with 1 ml storage buffer three times.
6. Suspend the beads with the desired volume of storage buffer and store at 4° C.

III. **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	