Cleavable Aldehyde-Activated Magnetic Beads

BcMag™ Cleavable Aldehyde-Activated Magnetic Beads are uniform, silica-based superparamagnetic beads grafted with a high density of cleavable aldehyde functional groups on the surface. The bead aldehyde groups react spontaneously with primary amines present at the N-terminus of proteins or in lysine residues to form intermediate Schiff Base complexes. The reaction of reductive amination immobilization starts with the creation of an initial Schiff base between the aldehyde and amine groups, which is then reduced to a secondary amine by the addition of sodium cyanoborohydride ($NaCNBH_3$) to generate stable amine bonds between the Bead and the ligand. At physiological to alkaline circumstances (pH 7.2 to 9) in either aqueous or organic solvents with 20- 30% DMSO or DMF, coupling reaction takes 2 to 6 hours in a one-step process. Coupling efficiency with antibodies and normal proteins is usually better than 85%, resulting in 15 - 20 µg /mg of beads.

The Cleavable Aldehyde-Activated Magnetic Beads are ideal for conjugating either larger protein or small peptides. Because the active aldehyde group is linked to the beads via a cleavable disulfide linker (Fig.1), reducing agents like DTT or -mercaptoethanol can cleave and separate the target molecule-ligand complex from the beads, leaving only a small sulfhydryl group attached to the ligand after affinity purification. Moreover, the hydrophilic surface ensures low nonspecific adsorption, excellent dispersion, and easy handling in various buffers.

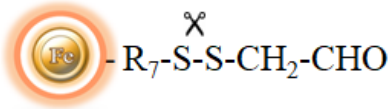

Fig.1

The Aldehyde-Activated resins have higher coupling efficiency than cyanogen bromide (CNBr) activated supports. Furthermore, the aldehyde Chemistry generates an uncharged connection with the amine-containing ligand that is more stable than the CNBr approach. The hydrophilic surface ensures excellent dispersion and easy handling in various buffers. When utilized for affinity purification methods, these features allow better leak-resistant immobilization and lower nonspecific binding.

Workflow (Fig.2)

The beads work perfectly as affinity resin for affinity purification to refine molecules, cells, and parts of cells into purified fractions. After conjugation with ligands, add the beads to a sample containing the target molecules, then mix, incubate, wash and elute the target molecules.

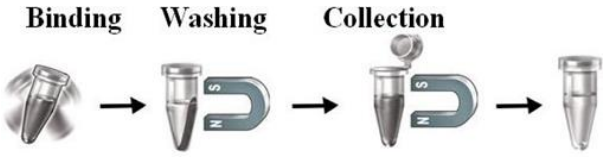

Fig.2

Features and benefits.

- Pre-activated and ready-to-use
- A cleavable built-in disulfide bond allows the ligand-target molecule complex to separate from the beads.
- Quick, Easy, and one-step high-throughput procedure; eliminates columns or filters or a laborious repeat of pipetting or centrifugation.
- Stable covalent bond with minimal ligand leakage
- High capacity—Immobilize 15 -20 µg antibody/mg beads.
- Scalable -easily adjusts for sample size and automation
- Low nonspecific binding
- Reproducible results
- Application: Purification for antibody, protein/peptide, DNA/RNA, cell sorting, immunoprecipitation

Specification		
Composition	Magnetic beads grafted with a high density of cleavable aldehyde groups.	
Number of Beads	• ~ 1.68 x 10^9 beads/mg (1µm beads) • ~1.47 x 10^8 beads/mg (2.5µm 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	~260 µmole / g of Beads
	2.5µm Magnetic Beads	~240 µmole / g of Beads
Storage	Ship at room temperature. Store at -20°C 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 reducing agents, tris, or other buffers containing primary amines or other nucleophiles because these will break the disulfide linker or compete with the intended coupling reaction. But the wash or storage buffers can have amino or carboxyl groups.*

Materials Required

1. Coupling Buffer

   Notes:

   - *Prepare buffer solution in a chemical fume hood because sodium cyanoborohydride is very toxic*.

   a. Soluble ligand coupling buffer : 0.1 M sodium phosphate, pH 7.0

   b. Insoluble ligand coupling buffer: 0.1 M sodium phosphate, pH 7, 10-30% acetone, dioxane, or alcohols, or dimethylformamide (DMF), or dimethylsulfoxide (DMSO) or 6 M Guanidine•HCl or 4 M Urea

2. Blocking Buffer: 1 M Tris•HCl, pH 7.4
3. Washing Buffer: 1 M NaCl
4. Sodium Cyanoborohydride Solution (5 M): NaCNBH3 (MW 62.84) dissolved in 1 M NaOH
5. 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).

Protein Coupling

A. Magnetic Beads Preparation

1. Prepare 3% magnetic beads with acetone (30 mg/ml) and mix well. 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.

   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 using the coupling buffer, use the Bead as soon as possible due to the stability of the functional group.*

B. Protein coupling

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1. Dissolve 0.5-10mg protein/peptide in 1ml soluble coupling buffer if soluble. If insoluble, dissolve in 1ml Insoluble coupling buffer. If samples have already been suspended in other buffers, dilute samples with a 4-fold volume of coupling buffer or desalt or dialyze to buffer-exchange into coupling buffer.

   Note:

   - *Coupling efficiencies to Aldehyde-activated magnetic beads vary from ligand to ligand. The user should empirically optimize the concentration of the ligand. We recommend 0.5-10 mg/ml for protein conjugation and at least 200 µmoles ligands per ml for small peptides.*

2. Add 100µl of the above protein solution and 10µl NaCNBH3 solution (10ul per milliliter of total volume, final concentration 50 mM) to the washed beads in a fume hood. Resuspend the magnetic beads and mix well by pipetting and incubate the reaction at room temperature or 4°C overnight with continuous rotation.
3. Wash beads three times with 1 ml coupling buffer.
4. Add 0.5-1ml blocking buffer and 5-10 µl $NaCNBH_3$ solution ((10ul per milliliter of total volume, final concentration 50 mM) to the beads and incubate in the room for 1 hour or at 4 *°C* overnight.
5. Wash beads 4-6 times with 1 ml washing buffer.
6. Resuspend the beads in PBS buffer with 0.01% 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. Designing a universal protocol for all protein purification is impossible because no two proteins are precisely alike. To obtain the best results, the user should determine the optimal working conditions for the purification of the individual target protein.*
- *Avoid reducing agents in the binding and washing buffers.*
- *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 temperature or desired temperature for 1-2 hours (Lower temperature 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) and 0.1-0.5% nonionic detergents such as Triton X100 or Tween20 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 SDS-PAGE sample buffer, or reducing agents.

7. Cleave the Disulfide Bond

   Note: *Due to conformational variation from ligands to ligands, the user should determine the optimal working conditions such as reducing agent, pH, and temperature for cleaving the disulfide bond of individual ligands. The following is an example of cleaving conjugated GFP from the beads.*

   1) Incubate the magnetic beads (30mg/ml) in either 140 mM β-mercaptoethanol or 5mM DTT (Dithiothreitol).

a. 100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 140 mM β-mercaptoethanol for 2 hours to overnight at room temperature or 98°C for 5 minutes.

b. 100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 5mM DTT for 2 hours to overnight at room temperature or 98°C for 5 minutes.

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
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