Cleavable Hydrazide-Terminated Magnetic Beads

BcMag™ Cleavable Hydrazide-Terminated Magnetic Beads are uniform magnetic beads grafted with a high density of cleavable hydrazide functional groups on the surface. Hydrazide chemistry is effective for labeling, immobilizing, or conjugating glycoproteins via glycosylation sites, which are frequently (as with most polyclonal antibodies) positioned in domains distant from the critical binding sites whose function needs to be preserved. Coupling antibodies in this manner selectively targets heavy chains in the Fc portion of the molecule, assisting in the best possible preservation of antigen binding activity by the ends of the Fv regions. At pH 5 to 7, hydrazide-terminated supports and compounds will conjugate with carbonyls of oxidized carbohydrates (sugars), producing hydrazone linkages.

The hydrazide beads are suitable for conjugating larger glycoproteins and glycolipids, carbohydrates or other ligands. Since the active hydrazide group is linked with the beads through a built-in cleavable disulfide linker (Fig.1), reducing agents such as DTT or β-mercaptoethanol can cleave separate the target molecule-ligand complex from the beads after affinity purification. Moreover, the hydrophilic surface ensures low nonspecific adsorption, excellent dispersion, and easy handling in various buffers.

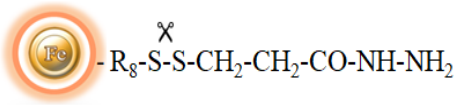

Fig.1 Cleavable hydrazide structure

Workflow

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)

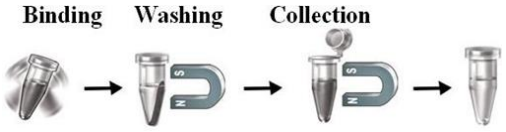

Fig.2

Features and benefits.

- Covalently coupling with a cleavable built-in disulfide bond allows the ligand-target molecule complex to separate from the beads.
- Specific immobilization—the hydrazide-activated beads bind exclusively to pure glycoproteins containing sugar groups that have been gently oxidized with periodate (e.g., sialic acid).
- Stable covalent bond with low levels of ligand leakage
- Maintains antibody function—immobilizes IgG via the Fc region, leaving both antigen binding sites available for target capture.
- Low nonspecific binding
- High capacity—Immobilize 15-20μg antibody/mg beads.

Specification	
Composition	Magnetic Bead grafted with cleavable hydrazide group on the surface.
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	~200 µmole / g of Beads
	2.5µm Magnetic Beads	~195 µmole/ g of Beads
Storage	Ship at room temperature. Store at 4°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. 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, pH 7.0
3. Oxidizing Agent: Sodium *meta*-Periodate ($NaIO_4$) (Sigma, Cat# S1878)
4. Washing Buffer: 1M NaCl
5. PBS buffer

Coupling

A. Beads preparation

1. Combine 30 mg beads with 1 ml coupling buffer.
2. Mix well by vortexing or pipetting.
3. 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.
4. The beads are ready for coupling.

B. Oxidation of Glycoprotein or other ligands

Note: *The reaction is light sensitive and should be performed in the dark.*

1. Dissolve or dilute 0.5-10 mg glycoprotein or other ligands in 1 ml coupling buffer.

   Note:

   If the protein or other ligands is already suspended in other buffers, perform a buffer exchange by dialysis or a desalting column.
2. Add the protein or other ligands solution to an amber vial containing 2 mg sodium meta-periodate (final concentration10mM). Swirl gently to dissolve the oxidizing agent.
3. Incubate the sample **in the dark** at room temperature for 45 minutes with good mixing (end-over-end).

C. Conjugation

Note: *Coupling efficiency depends on the structure and the size of the target glycoprotein or other ligands. The user should empirically optimize the ratio of the protein or other ligands to the beads.*

1. Add the oxidized protein or other ligands solution to the prepared magnetic beads and mix well by vortexing or pipetting.
2. Incubate the sample **in the dark** at room temperature overnight with good mixing (en-over-end).
3. Wash the beads with 5ml of washing buffer three times by a magnet.
4. Wash the beads with 5ml PBS
5. Resuspend the beads with PBS buffer containing 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. The user should determine the optimal working conditions for purifying the individual target protein to obtain the best results.*
- *Avoid reducing agents in all the 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 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) 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
Monomer Avidin Magnetic Beads	