

## Glycoprotein & Antibody conjugation kit (II)

### Introduction

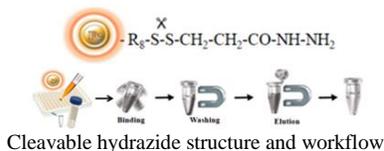
BcMag™ Glycoprotein & Antibody conjugation kit enables the formation of immobilization sites in biological compounds using carbonyl ketones or aldehydes present in similar groups on proteins. These sites drive covalent coupling away from active centers or binding regions. Glycoconjugates like glycoproteins and glycolipids contain sugar residues with hydroxyls on neighboring carbon atoms. Oxidizing these cis diols with sodium periodate results in aldehydes that serve as covalent immobilization sites.

The kit produces affinity support with glycoproteins and antibodies using hydrazide-activated magnetic beads and a specific catalyst. Monoclonal antibodies and glycosylated proteins can be immobilized using periodate-oxidizable carbohydrate groups to create reusable affinity matrices. This hydrazide immobilization chemistry yields antibodies with unobstructed antigen-binding sites and excellent purification capacity. The full coupling reaction takes 2 to 4 hours in a simple non-amine buffer containing the catalyst. Antibodies and common glycoproteins show coupling efficiency typically greater than 85%, yielding 15µg to 20 µg of immobilized protein per mg of the hydrazide-terminated magnetic beads. The matrix prepared with stable glycoproteins or antibodies can be regenerated and reused at least five times without appreciable loss of binding capacity.



Fig.1 Hydrazide coupling reaction

The **BcMag™ Cleavable Hydrazide-Terminated Magnetic Beads** are 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 that are frequently 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. Hydrazide-terminated supports and compounds conjugate with carbonyls of oxidized carbohydrates (sugars) to produce hydrazone linkages at pH 5 to 7. The hydrazide beads are suitable for conjugating larger glycoproteins and glycolipids, carbohydrates, or other ligands. The hydrophilic surface ensures low nonspecific adsorption, excellent dispersion, and easy handling in various buffers.



### 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.

### Features and benefits.

- 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 15-20 $\mu$ g antibody/mg beads.

Components	Storage	
2.5 $\mu$ m BcMag™ Cleavable Hydrazide-Terminated Magnetic Beads	4°C	150 mg
10x coupling Buffer	4°C	10 ml
Oxidizing Agent (NaIO <sub>4</sub> )	4°C	100 mg
5 x Wash Buffer	4°C	15 ml
10x PBS	4°C	5.0 ml

**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.

**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<sub>4</sub>) (Sigma, Cat# S1878)
4. Washing Buffer: 1M NaCl
5. PBS buffer
6. 2.5  $\mu$ m BcMag™ Cleavable Hydrazide-Terminated Magnetic Beads

**A. Beads preparation**

1. Weight 30 mg beads and add them into a 1.5 ml centrifuge tube.
2. Add 1 ml coupling buffer and resuspend the beads very 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 concentration 10mM). 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**

1. Add the oxidized protein or other ligands solution to the prepared magnetic beads from Step A4 and mix well by vortexing or pipetting.

**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 to the beads.*

2. Incubate the reaction **in the dark** at room temperature overnight with good mixing (end-over-end).



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. Remove the tube from the rack and resuspend the beads with 5 ml Coupling Buffer by vortex or pipette.
4. Repeat steps 3 for three times.
5. 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.

**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, the user should determine the optimal working conditions for the purification of the individual target protein.*
- *Avoid reducing agents in 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 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 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 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, the addition of NaCl (up to 1-1.5 M), 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.
7. For cleavable beads, 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 for 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.



<b>Related Products</b>	
Glycoprotein and Antibody Conjugation Kit-I	Peptide conjugation buffer Kit-I
Glycoprotein and Antibody Conjugation Kit-II	Peptide conjugation buffer Kit-II
Protein A and G Purification Kit	Quick Albumin Removal Kit
Protein A Magnetic Beads Purification Kit	Quick HSA and IgG Depletion Kit
Protein G Magnetic Beads Purification Kit	Quick Antibody Purification Kit
Protein L Purification Kit	One-Step Antibody Purification Kit
Protein A and G Europium Fluorescent Magnetic Beads	Protein G Europium Fluorescent Magnetic Beads
Protein A and G Terbium Fluorescent Magnetic Beads	Protein G Terbium Fluorescent Magnetic Beads
Protein A and G Ruthenium Fluorescent Magnetic Beads	Protein G Ruthenium Fluorescent Magnetic Beads
Protein A Europium Fluorescent Magnetic Beads	Protein L Europium Fluorescent Magnetic Beads
Protein A Terbium Fluorescent Magnetic Beads	Protein L Terbium Fluorescent Magnetic Beads
Protein A Ruthenium Fluorescent Magnetic Beads	Protein L-Ruthenium Fluorescent Magnetic Beads