

Peptide conjugation kit (II)

Introduction

It is frequently helpful to immobilize peptides via functional groups other than amines. The thiol group, in particular, can be employed to guide coupling processes away from active centers or binding sites on specific protein molecules. Using a unique active group for site-directed immobilization is beneficial for correct orientation, such as sulfhydryl on a single terminal cysteine in a peptide for a small antigen.

BcMag™ Peptide conjugation kit is designed to enable fast, efficient, and covalent immobilization of small peptides through their sulfhydryl groups (-SH) for affinity purification procedures. BcMag™ Cleavable Iodoacetyl-activated Magnetic Beads are recommended conjugation of small peptides because the long-arm hydrophilic linker may reduce steric hindrance. Since the active iodoacetyl 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 and separate the target molecule-ligand complex from the beads, and only a small sulfhydryl group is attached to ligand after affinity purification.

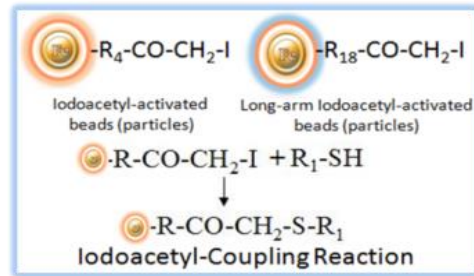


Fig.1 Iodoacetyl-sulfhydryl group coupling reaction

BcMag™ Cleavable Iodoacetyl-activated Magnetic Beads (Fig.2) are uniform and silica-based superparamagnetic beads coated with a high density of Iodoacetyl or cleavable Iodoacetyl functional groups on the surface. At physiological to alkaline circumstances (pH 7.2 to 9) in either aqueous or organic solvents 20- 30% DMSO or DMF, iodoacetyl-activated supports react with sulfhydryl groups, resulting in stable thioether bonds. These reactions are often carried out in the dark to prevent the formation of free iodine, which can react with tyrosine, histidine, and tryptophan residues.

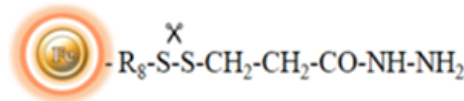


Fig2. Structure of cleavable hydrazide beads

Workflow

The Iodoacetyl magnetic beads work perfectly as solid support for various bioseparations 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.3)

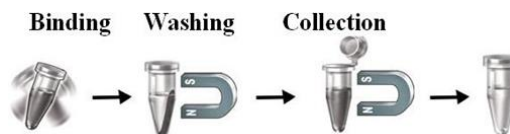


Fig.3 Workflow of Iodoacetyl-activated Magnetic Beads

Features and benefits.



- Iodoacetyl groups react selectively with sulfhydryl (-SH) groups to produce irreversible thioether linkages.
- Fast—couple peptide samples in 2 hours.
- Versatile coupling conditions—as needed for protein or peptide solubility during the coupling reaction, employ pH 7.5 to 9.0 aqueous buffers, organic solvent (e.g., 20% DMSO), or denaturant (guanidine-HCl).
- Simple to follow protocols for sample preparation, immobilization, and affinity purification
- High capacity—Immobilize 15 -20 µg antibody/mg beads.

Applications:

- Immobilize peptides with terminal cysteine residues to purify antibodies raised against peptide immunogens.
- Immobilize antibodies in an orientated manner using hinge-region sulfhydryl's to ensure that antigen binding sites are not sterically inhibited when antigen affinity purification is performed.
- Produces reusable immunoaffinity matrices
- Maintains antibody function—immobilizes IgG via the Fc region, leaving both antigen binding sites available for target capture.

Components	Storage	
2.5 µm Cleavable Iodoacetyl-activated Magnetic Beads	-20°C	150 mg
10x coupling Buffer	4°C	10 ml
Blocking Buffer (L-Cysteine•HCl)	4°C	100 mg
5 x Wash Buffer	4°C	15 ml
TCEP	-20°C	125 mg

Protocol

Note:

- This protocol can be scaled up as needed. We strongly recommended titration to optimize the number of beads used for each application.

Materials Required

- Coupling Buffer
 1. Soluble coupling buffer: 50 mM Tris, 5 mM EDTA-Na, pH 8.5;
 2. Insoluble coupling buffer: 50 mM Tris, 5 mM EDTA-Na, pH 8.5, 20- 30% DMSO or DMF or 6 M guanidine•HCl
- Wash Buffer: 1 M sodium chloride (NaCl) in distilled H₂O
- L-Cysteine•HCl
- TCEP (tris(2-carboxyethyl)phosphine)
- Phosphate buffered saline (PBS)
- BcMag™ Iodoacetyl-activated Magnetic Beads: The user can choose different Iodoacetyl-activated Magnetic Beads based on the requirements: 1µm BcMag™ Long-arm Iodoacetyl-activated Magnetic Beads, 2.5µm Long-arm Iodoacetyl-activated Magnetic Beads, 5µm BcMag™ Long-arm Iodoacetyl-activated, 1µm BcMag™ Cleavable Iodoacetyl-activated Magnetic Beads, or, 2.5µm BcMag™ Cleavable Iodoacetyl-activated Magnetic Beads.
- Magnetic rack

Based on sample volume, the user can choose one of the following magnetic Racks:

1. BcMag™ rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01)
2. BcMag™ rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02)
3. BcMag™ rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03)
4. BcMag™ rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)



5. BcMag™ 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible racks (Bioclone, Cat#: MS-06)

A. Ligand préparation

Note:

- *Ensure that ligands have free (reduced) sulfhydryls. If free sulfhydryl groups are not available, use a reducing agent such as DTT (dithiothreitol), TCEP (tris(2-carboxyethyl) phosphine), or 2-MEA (2-Mercaptoethylamine•HCl) to treat ligands followed by desalting or dialysis to remove the reducing agent.*
- *Newly Synthesized peptides may be directly used for coupling if used immediately after reconstitution.*
- *For protein, treat protein with 5-10 mM TCEP solution for 30 minutes at room temperature, followed by dialysis or a desalting column. For IgG antibodies, 2-MEA is recommended due to its Selective reduction of hinge-region disulfide bonds.*
- *If the sample contains reducing agents with free sulfhydryls (e.g., 2-mercaptoethanol or DTT), these agents must be completely removed by dialysis or desalting.*

1. Dissolve 1-10mg protein/peptide in 1ml soluble coupling buffer if soluble. If insoluble, dissolve in 1ml insoluble coupling buffer.
2. If samples have already been suspended in other buffers, dilute samples with an equal volume of coupling buffer.

B. Magnetic 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 has been 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 using the coupling buffer, use the bead as soon as possible due to the stability of the functional group.*

C. Coupling

1. Add 100 µl of ligand to the washed beads, mix well and incubate the sample in the dark at room temperature overnight with good mixing (en-over-end).
2. Wash the magnetic beads with 1ml coupling buffer four times.
3. Block the excess active groups on the beads by suspending the beads in 1ml Coupling buffer containing 8mg L-Cysteine•HCl and incubate 30-60 minutes at room temperature with gentle rotation.
4. Wash the beads with 1ml washing buffer four times.
5. Resuspend the beads in PBS buffer containing 0.05% sodium azide and store them at 4°C.

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 purifying 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 of 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. 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 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	
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