Iodoacetyl-activated Magnetic Beads

It is frequently helpful to immobilize affinity ligands 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.

Cysteines are frequently bonded between their side chains via disulfide bonds (-S-S-) as part of a protein's secondary or tertiary structure. The side chain of cysteine contains sulfhydryls (-SH) (Cys, C). These must be converted to sulfhydryls before being immobilized. Thiol groups (sulfhydryls) can be found naturally in proteins or introduced through the reduction of disulfides or using various thiolation reagents.

BcMag™ Iodoacetyl-activated Magnetic Beads are uniform, silica-based superparamagnetic beads coated with a high density of Iodoacetyl functional groups on the surface. It is designed to enable fast, efficient, and covalent immobilization of protein, peptides, and other ligands through their sulfhydryl groups (-SH) for affinity purification procedures. At physiological to alkaline circumstances (pH 7.2 to 9)) in either aqueous or organic solvents with 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 (Fig.1). The hydrophilic surface ensures beads low nonspecific adsorption, excellent dispersion, and easy handling in various buffers. BcMag™ Iodoacetyl-activated Magnetic Beads are most suitable for conjugation of a larger protein. BcMag™ Long-arm Iodoacetyl-activated Magnetic Beads are recommended to conjugate small peptides because the long-arm (20-atom) hydrophilic linker may reduce steric hindrance.

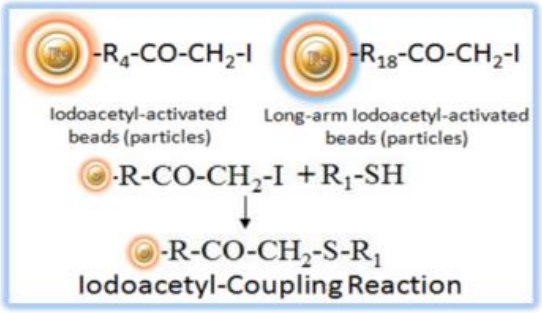

Fig.1 Iodoacetyl-sulfhydryl group coupling reaction

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.2)

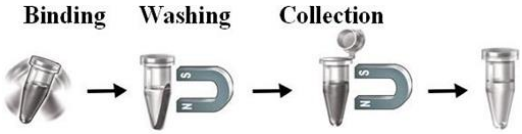

Fig.2

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 in 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 sulfhydryls 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.

Specification		
Composition	Magnetic grafted with a high density of iodoacetyl group.	
Number of Beads	~ 1.68 x 10^9 beads/mg (1µm beads) ~ 5 x 10^7 beads/mg (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
	5µm Magnetic Beads	~180 µmole / g of Beads
	1µm Long-Arm Magnetic Beads	~160 µmole / g of Beads
	5µm Long-Arm Magnetic Beads	~130 µmole / g of Beads
Storage	Store beads at -20°C protected from light and free of moisture 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.

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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 H_2O
- L-Cysteine•HCl
- TCEP (tris(2-carboxyethyl)phosphine)
- Phosphate buffered saline (PBS)
- 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 (Blioclone, Cat#: MS-06)

A. Ligand preparation

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℃. 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, 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	