Cleavable Iodoacetyl-activated Magnetic Beads

BcMag™ Cleavable Iodoacetyl-activated Magnetic Beads are uniform, silica-based superparamagnetic beads coated with a high density of cleavable 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 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. 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. Moreover, the hydrophilic surface ensures low nonspecific adsorption, excellent dispersion, and easy handling in various buffers. The beads are suitable for conjugating larger protein or small biomolecules without steric hindrance.

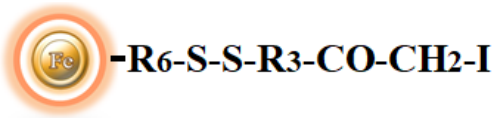

Fig.1 Structure of Cleavable Iodoacetyl-activated beads.

The iodoacetyl 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

- Iodoacetyl groups react selectively with sulfhydryl (-SH) groups to produce irreversible thioether linkages.
- Fast—couple peptide samples in 2 hours.
- A cleavable built-in disulfide bond allows the ligand-target molecule complex to separate from the beads.
- 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 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 cleavable iodoacetyl group.
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	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.*
- *Avoid reducing agents in the coupling, washing, and storage buffer.*

.**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. Ligagnd 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 completely.*
- *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. Prepare 100 µl of protein solution (0.5-1mg/ml) or peptide solution (200 µmoles/ml) with coupling buffe.
2. If samples have already been suspended in another buffer, 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 is stable for over a year.*
2. Transfer 100 µl (3mg) magnetic beads to a centrifuge tube.

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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 10 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.

Coupling

Note: ***Coupling efficiencies to iodoacetyl-activated magnetic beads varies from ligand to ligand. The user should empirically optimize the concentration of the ligand. R****ecommend* ***0.5-10 mg/ml for protein conjugation, 200µmoles/ml for small peptides.***

1. Mix the ligands with the washed beads by pipette and incubate the sample in the dark at room temperature overnight with good mixing (end-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. The user should determine the optimal working conditions for purifying the individual target protein to obtain the best results.*
- *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 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
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