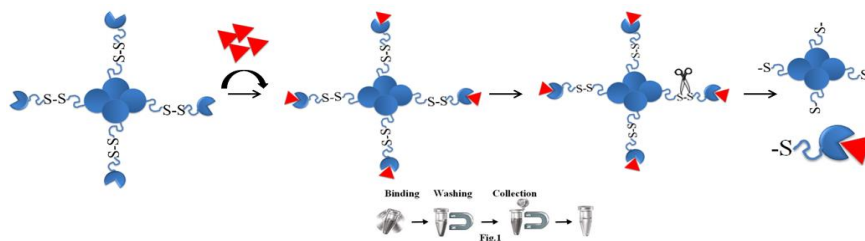


Cleavable Streptavidin Magnetic Beads

Introduction

BcMag™ Cleavable Streptavidin Magnetic Beads are uniform superparamagnetic microspheres coated with high purity of (>97%) streptavidin. Since the streptavidin is linked to the beads (particles) through a built-in cleavable disulfide linker, the reducing agents such as DTT or β -mercaptoethanol can cleave and separate the biotinylated molecule-streptavidin complex from the beads instead of harsh elution reagents such as 8M guanidine or SDS detergent after affinity purification (Fig.1). The microspheres are specifically designed and tested for applications in immunoprecipitation, cell sorting, and rapid single-step capture of biotinylated molecules such as DNA, RNA, antibody, or protein from the cell.

The interaction between streptavidin (or avidin) and biotin exhibits one of the highest known non-covalent interactions. Avidin, streptavidin, monomeric avidin, and their analogs have become powerful tools for probes and affinity ligands for various applications in biochemical assays, diagnosis, affinity purification, and drug delivery. Streptavidin is a tetrameric biotin-binding protein that originates from *Streptomyces* and has a mass of 60,000 Daltons. Streptavidin has no carbohydrate and lower pI, giving a lower degree of nonspecific binding, making streptavidin an ideal reagent choice for many detection systems.



Features and Advantages:

Advantages and benefits of using streptavidin biotin-binding systems.

- **High affinity**
Streptavidin is homo-tetramer and has a higher high biotin-binding affinity, with a $K_D \sim 10^{-14}$ M.
- **High stability**
The biotin-binding complex is exceptional stability and can resist Extreme pH, temperature (2 °C and 40 °C), harsh organic solvents, denaturing agents such as guanidinium chloride, detergents such as SDS and proteolytic enzymes.
- **Outstanding selectivity and specificity**
The interaction of the biotin and streptavidin is highly specific, ensuring low nonspecific binding.
- **High sensitivity**
High stability and specificity ensure high detection sensitivity.
- **Very flexibility**
The small size and remarkable stability of biotin are proven to be exceptionally suitable for relatively easy incorporation into various molecules such as synthetic polymers, fluorophores, small molecules, or into specific locations in biomolecules that impose minimal perturbation to the structure and function of the conjugated biomolecules.

Advantages and benefits of using streptavidin magnetic microspheres.

- Easily release the biotinylated molecule-streptavidin complex from the particles
- Quick, Easy, and one-step high throughput procedure eliminates Columns or filters or a laborious repeat of pipetting or centrifugation.
- High binding capacity
- Exhibits low nonspecific binding.
- Scalable - easily adjusts for sample size and automation.



Specification		
Bead Size	2.5µm diameter	
Number of Beads	~10 x 10 ⁷ particles/mg	
Magnetization	~40-45 EMU/g	
Type of Magnetization	Superparamagnetic	
Concentration	10 mg/ml (10mM Tris, 0.15 M NaCl, 0.1% BSA, 1 mM EDTA, pH7.4)	
Binding Capacity	Biotinylated BSA / ml of Beads	>1 mg/ml
	Biotinylate single-stranded oligonucleotides	~ 2,000 pmoles /ml
Storage	Ship at room temperature. Store at 4°C. Do not freeze	

Protocol

Materials Required

- Binding Buffer: 1x PBS, 0.1% BSA, pH 7.4
- 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).

Procedure

Note:

- *This protocol is a general affinity purification procedure. To obtain the best results, each user should determine the optimal working conditions for the purification of the individual target protein.*
- *We strongly recommend titration to optimize the number of particles used for each application based on the amount of the target biotinylated molecules in the crude sample based on the particles binding capacity. Too many magnetic particles used will cause higher backgrounds, while too few particles used will cause lower yields.*

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 molecules 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, the addition of NaCl (up to 1-1.5 M), 0.1-0.5% nonionic detergents such as Triton X 100 or Tween 20 to the washing buffer.*

6. 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