

Magnetic Beads Make Things Simple

Streptavidin Magnetic Beads

Introduction

BcMagTM Streptavidin Magnetic Bead is a highly uniform and superparamagnetic microsphere coated with a high density of high purity (>97%) streptavidin on the surface. The beads are manufactured using nanometer-scale superparamagnetic iron oxide as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide. The microspheres are specifically designed and tested for applications in immunoprecipitation, cell sorting, and rapid single-step capture of the target molecules such as DNA, RNA, antibody, or protein from cell lysates or hybridization reactions.

The interaction between streptavidin (or avidin n) 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 a wide range of applications in biochemical assays, diagnosis, affinity purification, and drug delivery. Streptavidin is a tetrameric biotin-binding protein that originates from *Streptomyces avidinii* 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 a variety of molecules such as synthetic polymers, fluorophores, small molecules, or into specific locations in biomolecules, that imposes minimal perturbation to the structure and function of the conjugated biomolecules.

Advantages and benefits of using streptavidin magnetic microspheres.

- Quick, Easy, and one-step high throughput procedure eliminates Columns or filters, or a laborious repeat of pipetting or centrifugation (Fig.1)
- High binding capacity
- Exhibits low nonspecific binding. Scalable easily adjusts for sample size and automation.



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Specification			
Bead Size	2.5µm diameter		
Number of Beads	$\sim 1.47 \text{ x } 10^8 \text{ beads/mg}$		
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	>1mg/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 recommended titration to optimize the number of beads used for each application based on the amount of the target biotinylated molecules in the crude sample based on the beads binding capacity. Too many magnetic beads used will cause higher backgrounds, while too few beads 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.
- 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.

Questions and Answers:

1. How is it possible to break the bond between biotin and streptavidin?



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The chemical bond between biotin and streptavidin is incredibly strong. It can break under denaturing conditions. Many applications do not require the separation of biotin from streptavidin. However, if the user needs to separate the biotin from streptavidin, follow the following suggestions:

• Protein or antibody

To elute the bound biotinylated protein or antibody from the beads, add the appropriate amount of Elution Buffer (0.1 M Glycine-HCl, pH 2.5), incubate for 5 min at room temperature, collect the magnetic beads by a magnetic rack and transfer the supernatant to a fresh tube. Immediately neutralize the supernatant to pH 7.0 by adding 1/10 volume of Neutralization Buffer (1.0 M Tris-HCl, pH 9.0).

• DNA

Long nucleic acids used as biotinylated probes are not recommended due to difficulty in elution. For short biotinylated oligos, the user can try the following method:

a. To elute the bound biotinylated oligo from the beads, add an appropriate amount of Elution Buffer (10 mM EDTA, pH 8.2, 95% formamide), incubate at 65° C for 2 min.

b. Collect the magnetic beads by the magnetic rack and transfer the supernatant to a fresh tube.

2. How can I elute the bound nonbiotinylated sample from the Magnetic Beads?

• Protein or antibody

If a non-biotinylated protein or antibody interacts with a biotinylated antibody or protein, add an appropriate amount of Elution Buffer (0.1 M Glycine-HCl, pH 2.5), incubate for 30 sec-5 min at room temperature, collect the magnetic beads by the magnetic rack and transfer the supernatant to a fresh tube. Immediately neutralize the supernatant to pH 7.0 by adding 1/10 volumes of Neutralization Buffer (1.0 M Tris-HCl, pH9.0).

• DNA or RNA

The biotinylated DNA or RNA is a short oligo probe. The bound non-biotinylated DNA or RNA can be eluted by adding an appropriate amount of dH_2O and heating at 65-70° C for 3-5 min. Collect the magnetic beads by the magnetic rack and transfer the supernatant to a fresh tube. The biotinylated DNA or RNA is a large fragment. The bound non-biotinylated DNA can be eluted by adding the appropriate amount of dH_2O and heating at 95° C for 3-5 min. Collect the magnetic beads by the magnet by the magneti

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