

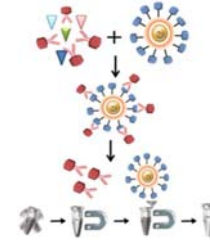
BcMag™ Streptavidin Magnetic Beads

Introduction

BcMag™ Streptavidin Magnetic Beads are 1 µm, uniform, silica-based superparamagnetic beads coated with high density ultrapure (>97%) streptavidin on the surface. The Beads are specifically designed, tested and quality controlled for use in immunoprecipitation, cell sorting, rapid single-step capture of biotinylated molecules such as DNA, RNA, antibody or protein from cell lysates or hybridization reactions.

Features and Advantages:

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



Composition	Silica-coated iron oxide magnetic beads Conjugated with streptavidin on the surface	
Bead Size	1µm diameter	
Number of Beads	~1.7 x 10 ⁸ beads	
Surface Area	~100 m ² /g	
Magnetization	~40-45 EMU/g	
Type of Magnetization	Superparamagnetic	
Effective Density	2.5 g/ml	
Concentration	10 mg/ml (10mM Tris, 0.15 M NaCl,0.1% BSA,1 mM EDTA, pH7.4, 0.01% NaN ₃)	
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	

Buffer Composition

- BcMag.Streptavidin Magnetic Beads: Suspended in 10 mM Tris, 0.15 M NaCl,0.1% BSA,1 mM EDTA, pH 7.4, 0.01% NaN₃

Materials Required

- **Magnetic Separator (for manual operation):** Based on sample volume, user can choose one of the following magnetic Separators: BcMag™ separator-2 for holding two individual 1.5 ml centrifuge tubes (Cat.# MS-01); BcMag™ separator-6 for holding six individual 1.5 ml centrifuge tubes (Cat.# MS-02); BcMag™ separator-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat.# MS-03); BcMag™ separator-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™ separator-96 for holding 96 ELISA plate (Cat.# MS-05).

Protocol

Application of BcMag.Streptavidin Magnetic Beads consists of two steps:

1. Biotinylation (conjugation of protein or nucleic acid with biotin); and 2. Immobilization (formation of the complex of biotinylated molecules with BcMag.Streptavidin Magnetic Beads).

A. Biotinylation

Note:

Before biotinylation, the user must be aware that:

1. In order to decrease steric hindrance, at least a 6 Carbon atom spacer arm is required between the biotin and the target molecule.
2. Free biotin should be removed from the solution after biotin labeling, because excess free biotin left in the solution will decrease the binding capacity of the BcMag.Streptavidin Magnetic Beads to the target molecules.
3. Biotinylated oligonucleotides should be further purified by reverse HPLC/FPLC after biotin labeling. In order for DNA to be extended at its 3 end during in vitro DNA synthesis, oligo DNA should be biotinylated at its 5-end.

A1. Protein or antibody

Materials to be supplied by user:

- The Biotin-N-Hydroxy succinimidobiotin (Pierce,Cat.No.20217)
- DMSO (Sigma, Cat. No. D2650)
- NaHCO₃
- Tris
- Glycine

Buffer Composition



- PBS Buffer: 137mM NaCl, 2.7mM KCl, 4.3mM Na₂HPO₄, 1.4mM KH₂PO₄, pH 7.5
- Reaction Buffer: 100mM NaHCO₃, pH 8.2
- Binding Buffer: 20mM Tris-HCl pH 7.5, 1mM EDTA, 2.0 M NaCl.
- Washing Buffer: 50mM Tris-HCl pH 7.5, 1mM EDTA, 1.0 M NaCl
- Protein Elution Buffer: 0.1 M Glycine-HCl, pH 2.5
- Oligo Elution Buffer: 10mM EDTA, pH 8.2 and 95% formamide
- Stop Buffer: 2.0 M Glycine
- Neutralization Buffer: 1.0 M Tris-HCl, pH 9.0

Procedure

1. Dissolve 0.1-1.0 mg protein or antibody in an appropriate buffer, such as PBS Buffer (phosphate buffered saline).
Note:
Do not dissolve protein or antibody in Tris or Glycine buffer. If the sample has been dissolved in Tris or Glycine buffer, the sample should be first dialyzed against 100 mM NaHCO₃, pH 8.2 to remove competing amine groups.
2. Add 1/10 volume of 1.0 M NaHCO₃, pH 8.2. Calculate the molar concentration of protein/antibody solution.
3. Dissolve 10x molar excess of biotin in 10 μ l DMSO and combine the biotin solution with the Protein/antibody solution.
4. Gently shake for 1 hr at room temperature with rotational mixing.
5. Add 1/20 volume of Stop Buffer to stop the biotinylation reaction.
6. Dialyze against 1x PBS Buffer for 24 hr at 4° C to remove free biotin. Change buffer 3 - 4 times during dialysis.
7. Calculate the final concentration of protein/antibody solution. Add BSA to a final concentration of 0.1%. Store at 4° C.

A2. DNA

- Biotinylated oligonucleotides can be purchased from commercial sources.
- Biotinylation of large DNA fragments
 1. Use 5 end biotinylated oligo primer for PCR.
 2. Incorporate biotin-dTTP/biotin-dATP into double strain DNA by nick translation or random labeling.

A3. RNA

- Incorporate biotin-UTP/biotin-ATP into RNA by *in vitro* transcription.
- Randomly incorporate biotin into RNA molecule by UV exposure

B. Immobilization

Note: *BcMag.Streptavidin Magnetic Beads* should be washed to remove 0.02% NaN₃ by the following steps before use.

1. Gently shake the bottle containing beads until the magnetic beads are completely suspended, and transfer appropriate amount of beads to a fresh tube.
2. Place the tube into a magnetic separator and wait for 2-3 min until supernatant becomes clear. Remove tube from the magnetic separator. Completely remove and discard the supernatant.
4. Add 1.0 ml RNase-free dH₂O. Remove the centrifuge tube from the magnetic separator. Mix well by gently pipetting several times. Put the tube back into the magnetic separator. Wait for 2-3 min until supernatant becomes clear. Remove tube from the magnetic separator. Completely remove and discard the supernatant.
5. Repeat (step 4) one more time. The beads are ready for use.

B1. Protein / Antibody or Nucleic acid

1. Combine 5.0 - 6.0 μ g biotinylated proteins/antibodies or 5.0 - 10 μ g biotinylated dsDNA or 100 pmoles biotinylated single-stranded oligonucleotides per 50 μ l of completely suspended BcMag. Streptavidin Beads with the pre-treated beads as described above.
2. Add 1.0 ml Binding Buffer (1x PBS, 0.1% BSA, pH 7.4) into the tube. Incubate for 30 min at room temperature with rotational mixing.
3. Place the tube into a magnetic separator, wait 1 - 3 min and completely remove and discard supernatant.
4. Add 1.0 ml Binding Buffer, remove the tube from the magnetic separator and mix well by inverting the tube several times. Place the tube into the magnetic separator, wait for 3 min. Completely remove and discard supernatant.
5. Repeat (step 4) four times.
6. Add appropriate amount of buffer to suspend the beads.
7. The beads are ready for desired use.



Questions and Answers:

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

The chemical bond between biotin and streptavidin is incredibly strong. It can only be broken under denaturing conditions. In fact, many applications do not require separation of biotin from streptavidin. However, if the user needs to separate the biotin from streptavidin, the following suggestions can be followed:

- **Protein or antibody:**

To elute the bound biotinylated protein or antibody from BcMag.Streptavidin Magnetic Beads, add the appropriate amount of Elution Buffer (0.1 M Glycine-HCl, pH 2.5), incubate for 0.5-5 min at room temperature, collect the magnetic beads by the magnetic separator 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, user can try the following method:

- a. To elute the bound biotinylated oligo from BcMag Streptavidin Magnetic Beads, add 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 separator and transfer the supernatant to a fresh tube.

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

1. Protein or antibody:

If a non-biotinylated protein or antibody interacts with biotinylated antibody or protein, add 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 separator 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).

2. 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 appropriate amount of d_2H_2O and heating at 65-70° C for 3-5 min. Collect the magnetic beads by the magnetic separator 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 appropriate amount of d_2H_2O and heating at 95° C for 3-5 min. Collect the magnetic beads by the magnetic separator and transfer the supernatant to a fresh tube.