

BcMag.Streptavidin Magnetic Beads

Introduction

BcMag.Streptavidin Magnetic Bead (1-2 μ m) is designed and used for rapid, single-step capture of biotinylated molecules such as DNA, RNA, antibody or protein from cell lysates or hybridization reactions. The beads are broadly stable over a pH range of 5.0 - 9.0 and at a temperature range of 4 - 80° C

Components

Cat. No.	MMI 105	MMI 106	MMI 107	MMI 108
Contents				
BcMag. streptavidin Magnetic Beads	5.0ml	10ml	20ml	50ml

Buffer Composition

- BcMag.Streptavidin Magnetic Beads: Suspended in 10 mM Tris, 0.15 M NaCl, 0.1% BSA, 1 mM EDTA, pH 7.4, 0.1% Na₂S₂O₃

Binding capacity

Type	Binding capacity (per ml of magnetic beads)
Free biotin	~ 4,000 pmoles
Biotinylated single stranded oligonucleotides	~ 2,000 pmoles

Storage: 4° C (Do not freeze)

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:

- In order to decrease steric hindrance, at least a 6 Carbon-atom spacer arm is required between the biotin and the target molecule.
- 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.
- 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

- 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 first be dialyzed against 100 mM NaHCO₃, pH 8.2 to remove competing amine groups.

- Add 1/10 volume of 1.0 M NaHCO₃, pH 8.2. Calculate the molar concentration of protein/antibody solution.
- Dissolve 10x molar excess of biotin in 10 μ l DMSO and combine the biotin solution with the protein/antibody solution.
- Gently shake for 1 hr at room temperature with rotational mixing.
- Add 1/20 volume of Stop Buffer to stop the biotinylation reaction.
- Dialyze against 1x PBS Buffer for 24 hr at 4° C to remove free biotin. Change buffer 3 - 4 times during dialysis.
- 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
- Use 5' end biotinylated oligo primer for PCR.
 - Incorporate biotin-dTTP/biotin-dATP into double strand 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% Na₂S₂O₃ by the following steps before use.

- Gently shake the bottle containing BcMag.Streptavidin Beads until the magnetic beads are completely suspended, and transfer the appropriate amount of beads to a fresh tube.
- Put 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.

Note: Magnetic separators are commercially available from BioClone Inc.: BcMag separator-2 for holding two individual 1.5 ml centrifuge tube, Cat. No. MS-01; BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes, Cat. No. MS-02;

BcMag separator-24 for holding twenty-four individual 1.5 ml centrifuge tubes, Cat. No. MS-03; BcMag separator-50 for holding one 50 ml and one 15 ml centrifuge tube, Cat. No. MS-04.

3. Add 1.0 ml RNase-free d_2H_2O . Take the centrifuge tube off 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.
4. Repeat (step 4) one more time. The beads are ready for use.

B1. Protein / Antibody or Nucleic acid

1. Combine 5.0 - 6.0 μg biotinylated proteins/antibodies or 5.0 - 10 μg biotinylated dsDNA or 100 pmoles biotinylated single-stranded oligonucleotides per 50 μl 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. Put the tube into a magnetic separator, wait 1 - 3 min and completely remove and discard supernatant.
4. Add 1.0 ml Binding Buffer, take the tube off the magnetic separator and mix well by inverting the tube several times. Put 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:

1. 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.
2. 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, pH 9.0).

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.