INSTRUCTIONS

MagnaBind™ Streptavidin Beads

21344 0723.4

Number	Description
21344	**MagnaBind Streptavidin Beads**, 5mL, supplied in phosphate-buffered saline (PBS), pH 7.5 containing 0.1% BSA, EDTA and sodium azide as stabilizers
	Binding Capacity: ~2µg biotin/mL of beads (~8nmol biotin/mL of beads)
	Storage: Upon receipt store product at 4°C. Do not freeze product. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific MagnaBind Streptavidin Beads are convenient for affinity purification or separation methods involving of biotin-labeled molecules. To remove the MagnaBind Beads from the suspension, an external magnetic field is used. General characteristics of MagnaBind Beads are listed in Table 1.

Streptavidin is a 60kDa protein from *Streptomycetes avidinii*.[1] The protein is a tetramer having four biotin-binding sites. Unlike avidin, streptvidin has a low isoelectric point (pI=5) and no carbohydrate groups, resulting in low nonspecific binding. The high-affinity interaction between streptavidin and biotin cannot be dissociated efficiently except with very harsh conditions, such as boiling in sample loading buffer for SDS-PAGE or 8M guanidine•HCl, pH 1.5. Consequently, it is often possible to elute binding partners in an interaction complex without also eluting the biotinylated component.

Table 1. Characteristics of non-derivatized Thermo Scientific MagnaBind Beads.

Composition:	Silanized iron oxide
Magnetization:	25-35EMU/g
Type of Magnetization:	Superparamagnetic (no magnetic memory)
Surface Area:	>100m^2/g
Bead Size:	1-4µm diameter
Settling Rate:	4% in 30 minutes
Effective Density:	2.5g/mL
Number of Beads:	1×10^8 beads/mg
pH Stability:	Aqueous solution, above pH 4.0
Concentration:	5mg/mL

Note: To establish a microbe-free preparation, the MagnaBind Beads may be gamma-irradiated or washed with antibiotic medium.

Important Product Information

- Do not freeze, dry or centrifuge MagnaBind Beads. Freezing, drying or centrifuging will cause the beads to aggregate and lose binding activity.
- A low-pH elution buffer is acceptable for single-use applications; however, using pH<4 will inactivate the beads and may result in leaching of streptavidin tetramers or monomers. For multiple use applications, use neutral pH elution conditions such as Gentle Ag/Ab Elution Buffer (see Related Thermo Scientific Products).
- Boiling the beads in SDS-PAGE sample buffer is acceptable for single-use applications; boiling will cause bead aggregation and loss of binding activity.

Procedure for Removing Biotinylated Protein from a Solution

A. Additional Materials Required

- 1.5mL microcentrifuge tubes
- MagnaBind Magnet for a 1.5mL Microcentrifuge Tube (Product No. 21357 or 21359)
- Phosphate-buffered saline (PBS) consisting of 100mM sodium phosphate, 150mM NaCl, pH 7.2 (Product No. 28372). Other physiological buffers may also be used, including Tris-buffered saline (TBS), Product No. 28379.
- Sample (50-1500µL) containing biotinylated proteins to be removed

B. Procedure

1. Shake the bottle of MagnaBind Streptavidin Beads to resuspend the beads; then pipette 1mL into a microcentrifuge tube.
2. Place the tube in the MagnaBind Magnet to separate the beads; when the supernatant becomes clear, gently aspirate the supernatant and discard it.
3. Remove the tube from the MagnaBind Magnet, add 1mL of PBS, close tube and invert several times to resuspend beads.
4. Place the tube in the MagnaBind Magnet to separate the beads; remove and discard the supernatant.
5. Repeat Steps 3 and 4 two additional times for a total of three washes.
6. Add the protein sample (containing an amount of biotinylated protein that can be bound by the amount of beads used) to the tube containing washed beads. Remove tube from magnet and invert several times to mix. Incubate at room temperature for 30 minutes with constant or periodic mixing.
7. Magnetically separate the beads; then remove and save the supernatant, which is now depleted of biotinylated protein.

   Note: Recovery of the biotinylated proteins that are bound to the MagnaBind Streptavidin Beads requires harsh conditions, such as boiling with sample loading buffer for SDS-PAGE or 8M guanidine•HCl, pH 1.5. Streptavidin protein may leach from the beads with such conditions.

Procedure for Immunoprecipitation Using a Biotinylated Antibody

A. Additional Materials Required

- 1.5mL microcentrifuge tubes
- MagnaBind Magnet for a 1.5mL Microcentrifuge Tube (Product No. 21357 or 21359)
- Biotinylated Antibody, 5-100µg, diluted in 50-1000µL of physiologic buffer. Use an amount that can be easily bound by the amount of beads used (e.g., <100µg antibody per milliliter of MagnaBind Streptavidin Bead Solution)
- Antigen-containing Sample: 50-1500µL in a buffer that is compatible with antibody binding
- Binding/Wash Buffer: Phosphate-buffered saline (PBS) consisting of 100mM sodium phosphate, 150mM NaCl, pH 7.2 (Product No. 28372). Other physiological buffers may also be used, including Tris-buffered saline (TBS), Product No. 28379. Carefully read information about Elution Buffers.
- Elution Buffer: The optimal elution buffer is both compatible with the beads (see Important Product Information) and effective in dissociating the antigen from the antibody.
    - Low pH elution buffers such as 0.1M glycine•HCl, pH 2.5-3.0 (IgG Elution Buffer, Product No. 21004) are effective for most antibody-antigen interactions. However, the low pH condition may cause streptavidin to leach from the beads, resulting in a less pure immunoprecipitation product and preventing reuse of the beads.
    - High salt, neutral pH elution buffers include 3.0M potassium chloride, 5.0M potassium iodide, 3.5M magnesium chloride or Thermo Scientific Gentle Ab/Ag Elution Buffer (Product No. 21027). These buffers can elute many antibody-antigen interactions and will not damage or strip the MagnaBind Beads. In fact, the biotinylated antibody will remain bound to the beads, possibly allowing the beads to be reused one or two more times without having to bind antibody again. However, the magnesium chloride and Gentle Ab/Ag Elution Buffer will precipitate with phosphate buffer; therefore, a non-phosphate buffer such as TBS must be used in the wash steps immediately before elution when these buffers will be used. Also, the eluted sample will have to be dialyzed or desalted to make the sample compatible for electrophoresis and other applications.

B. Procedure

1. Shake the bottle of MagnaBind Streptavidin Beads to evenly resuspend the beads; then pipette 0.25-1mL into a microcentrifuge tube.
2. Place the tube in the MagnaBind Magnet to separate the beads; when the supernatant becomes clear, gently aspirate the supernatant and discard it.
3. Add 1mL of Binding/Wash Buffer, remove tube from magnet, and invert the tube several times to resuspend the beads. Then magnetically separate the beads, and remove and discard the supernatant.
4. Repeat Step 3 two additional times for a total of three washes.
5. Add 5-100µg of Biotinylated Antibody, remove tube from magnet, and gently invert tube several times to mix. Incubate at room temperature for 30 minutes with constant or periodic mixing.
6. Place the tube in the MagnaBind Magnet to separate the beads; remove the supernatant, which contains any antibody that did not bind to the beads.
7. Add 0.1-1.5mL Antigen Sample, remove tube from magnet, and gently invert tube several times to mix. Incubate at room temperature for 30 minutes with constant or periodic mixing.
8. Magnetically separate the beads and remove the supernatant, which contains nonbound components of the sample.
9. Add 1mL of Binding/Wash Buffer, remove tube from magnet, and invert the tube several times to resuspend the beads. Then magnetically separate the beads and remove and discard the supernatant.
10. Repeat Step 9 two additional times for a total of three washes. If the Gentle Ab/Ag Elution Buffer or 3.5M magnesium chloride will be used for elution, then use a non-phosphate buffer for at least the last one of these wash steps.
11. Add a small volume (typically 30-50µL) of Elution Buffer, remove tube from magnet, and invert or gently vortex the tube several times to resuspend the beads in the buffer. Then magnetically separate the beads and remove the solution, which is an elution fraction containing the immunoprecipitated antigen.
12. Repeat Step 11 two additional times for a total of three elution fractions.
13. Analyze the elution fractions. If a high salt elution buffer was used, dialyze or desalt the sample before attempting to mix with loading buffer for SDS-PAGE.

    Note: To reuse the antibody-bound beads, wash them several times in Binding/Wash Buffer before storage or addition of another antigen-containing sample. It is not possible to strip off the bound biotinylated antibody and reuse the beads with a different biotinylated antibody.

Related Thermo Scientific Products

21358	**MagnaBind™ Magnet for 96-Well Separator,** 1 each
21357	**MagnaBind Magnet for one 1.5mL Microcentrifuge Tube,** 1 each
21359	**MagnaBind Magnet for 6 Microcentrifuge Tubes,** 1 each
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
28379	**BupH Tris Buffered Saline Packs,** 10 packs
21027	**Gentle Ag/Ab Elution Buffer,** 500mL
21004	**IgG Elution Buffer,** 1L
69576	**Slide-A-Lyzer® MINI Dialysis Units, 10K MWCO, 0.1mL**
89882	**Zeba™ Spin Desalting Columns, 7K MWCO, 0.5mL,**
21955	**EZ-Link™ Micro NHS-PEG$_4$-Biotinylation Kit**

Cited Reference

1. Chaiet, I. and Wolf, F.J. (1964). The properties of streptavidin, a biotin-binding protein produced by *Streptomycetes*. *Arch Biochem Biophys* **106:**1-5.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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