

TECHNICAL MANUAL

High Capacity Magne[®] Streptavidin Beads

Instructions for Use of Product
V7820



High Capacity Magne[®] Streptavidin Beads

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1. Description

High Capacity Magne[®] Streptavidin Beads are magnetic affinity beads with high specificity and high capacity for binding biotinylated antibodies and proteins. The magnetic beads are composed of iron oxide encapsulated by macroporous cellulose, which results in beads that have low nonspecific binding. This makes High Capacity Magne[®] Streptavidin Beads ideal for use with complex biological samples.

High Capacity Magne[®] Streptavidin Beads can be used for a variety of applications including pharmacokinetics studies of therapeutic antibodies. Biotinylated antibodies (e.g., Goat Anti-Human Biotinylated IgG, Cat.# V7830) or antigens immobilized on High Capacity Magne[®] Streptavidin Beads can enrich therapeutic antibodies from serum or plasma, thus providing high purity samples for analysis by mass spectrometry. The high capacity of the beads enables enrichment of antibodies over a wide concentration range using a small amount of beads. The affinity of streptavidin for biotin ($K_d = 10^{-15}M$) is one of the strongest, most stable interactions in biology; this interaction cannot be reversed under nondenaturing conditions. Hence, it is possible to perform extensive washing to remove nonspecifically bound protein and elute therapeutic antibodies without eluting the biotinylated component, thereby improving the detection limit of the antibody.

The beads also have excellent magnetic properties for rapid and efficient capture using a variety of magnetic stands (see Section 6, Related Products). The capture process can be automated for high throughput and scaled up to handle a variety of sample volumes.

Table 1. High Capacity Magne[®] Streptavidin Bead Characteristics.

Composition	Magnetic bead based on macroporous cellulose
Chemistry	Covalent attachment of streptavidin
Particle Size	30–50 μ m
Biotinylated Antibody Binding Capacity on the Beads	≥ 9 mg of Goat Anti-Human Biotinylated IgG/ml of settled resin or ≥ 1.8 mg of Goat Anti-Human Biotinylated IgG/ml of bead slurry
Bead Formulation	20% slurry in 10mM phosphate buffer + 0.02% sodium azide

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
High Capacity Magne [®] Streptavidin Beads	3ml*	V7820

*Provided as a 20% slurry.

Storage Conditions: Store the High Capacity Magne[®] Streptavidin Beads at 2–10°C. Do not freeze. Do not allow beads to dry during storage or use. Do not reuse the beads.

3. Immuno-Enrichment Protocol

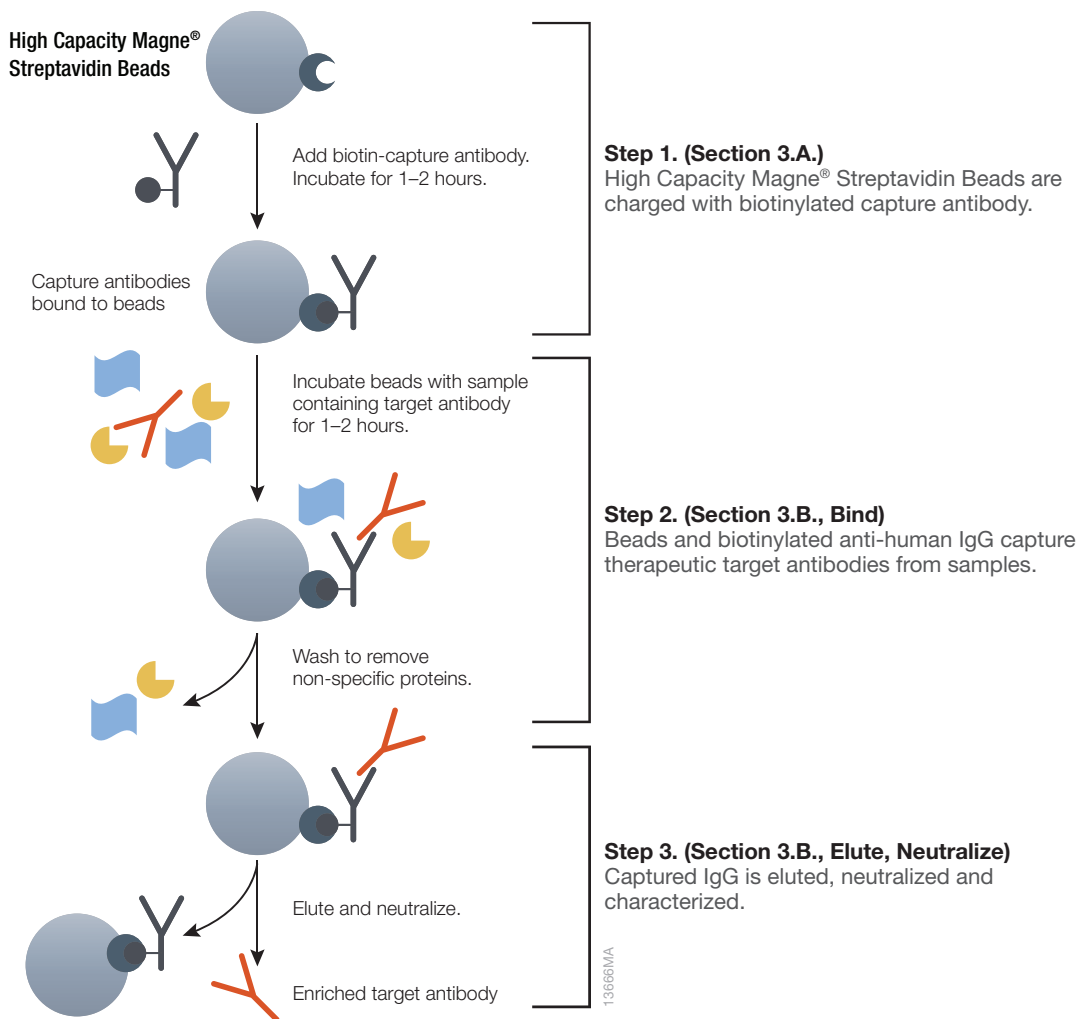


Figure 1. Schematic of immuno-enrichment protocol using High Capacity Magne® Streptavidin Beads.



3. Immuno-Enrichment Protocol (continued)

The following is a suggested protocol for enrichment of therapeutic antibody containing a human Fc region from a non-primate plasma or sera matrix. The protocol is for a single reaction using 25 μ l of streptavidin bead slurry charged with Goat Anti-Human Biotinylated IgG (Cat.# V7830), followed by enrichment of human antibody from 25 μ l of non-primate plasma or sera samples. As a general guideline, a 25 μ l slurry of High Capacity Magne[®] Streptavidin Beads will bind at least 45 μ g of Goat Anti-Human Biotinylated IgG, which in turn will capture at least 15 μ g of purified human IgG. We recommend that you optimize bead charging as well as the volume of beads used, based on lot-specific information on bead capacity and the expected amount of human IgG in the sample.

Notes:

1. For high-throughput applications using a 96-well plate, the use of a robotic system (e.g., Tecan, Beckman Coulter, Hamilton) is recommended. For assistance in adapting the protocol to a variety of automated platforms contact Promega Technical Services (techserv@promega.com).
2. For optimal binding be sure that the High Capacity Magne[®] Streptavidin Beads remain in suspension during binding and wash steps. To prevent settling of the beads we recommend using a tube shaker (e.g., Eppendorf Thermomixer, 1,100rpm) or end-over-end mixer.
3. Sample incubation times may need to be optimized. Binding can be performed at 4°C; however, longer incubation times may be necessary for efficient antibody capture when performed at 4°C.

Materials to Be Supplied by the User

(Solution compositions are provided in Section 5.)

- Goat Anti-Human Biotinylated IgG (Cat.# V7830)
- bind/wash buffer
- elution buffer
- neutralization buffer
- magnetic stand
- mixing platform, tube shaker or end-over-end mixer

3.A. Charge High Capacity Magne[®] Streptavidin Beads with Goat Anti-Human Biotinylated IgG

Equilibrate the Beads

1. Gently vortex or invert the tube containing the beads to obtain a uniform suspension and re-mix as necessary to maintain uniform suspension of beads when making aliquots.
2. Add 25 μ l of bead slurry (5 μ l of settled bed volume) to a 1.5ml microcentrifuge tube. This amount of bead slurry is sufficient for one immuno-enrichment reaction. Scale up the reaction as needed.
3. Place the tube in the magnetic stand for 10 seconds. Remove and discard the storage buffer.

Bind Anti-Human Biotinylated IgG

4. Add 250µl of bind/wash buffer (Tris buffered saline [pH 7.2]).
5. Mix gently and place the tube in the magnetic stand for 10 seconds.
6. Remove the bind/wash buffer from the tube and discard the buffer.
7. Add 100µl of Goat Anti-Human Biotinylated IgG (50µg). **Note:** This amount of antibody will completely saturate the streptavidin beads with a capacity of approximately 9.0mg/ml of beads. Adjust the amount of biotinylated antibody used for charging based on the actual lot-specific bead capacity.
8. Mix the sample for 120 minutes at room temperature, ensuring the beads remain in suspension. (We recommend a rotary mixer or Eppendorf vortex mixer at a speed of at least 1,100rpm.)
9. Place the tube on the magnetic stand for 10 seconds.
10. Remove the supernatant and measure absorbance of the flow through at 280nm (A_{280} (FT)) along with absorbance at 280nm for the starting material (A_{280} (SM)).
11. Calculate the amount of biotinylated antibody bound to the 100µl of bead slurry as shown below. (The expected amount is approximately 50µg of antibody per 25µl of bead slurry.)

Amount of antibody on the beads (25µl of slurry) = $[A_{280}$ (SM) - A_{280} (FT)] ÷ 1.4 × volume of biotinylated Ab,
where 1.4 absorbance units = 1mg/ml.

This value is for antibodies only. Adjust the calculation for different proteins.

Wash

12. Add 250µl of bind/wash buffer to the tube and mix for 5 minutes.
13. Place in the magnetic stand for 10 seconds.
14. Remove and discard the buffer. Repeat two more times for a total of 3 washes.
15. Add 20µl of bind/wash buffer to make 25µl of 20% slurry of magnetic beads charged with Goat Anti-Human Biotinylated IgG.
16. Use the beads for immuno-enrichment immediately, or store at 4°C for later use.



3.B. Capture Human IgG from Non-Primate Serum Sample

Bind

1. Dilute 25 μ l of serum sample in 200 μ l of bind/wash buffer. In this example, the serum sample must be non-primate serum containing human IgG, as will be the case during pharmacokinetic studies of human IgG in rodents.
2. Add the diluted sample to the microcentrifuge tube from Step 16 (Section 3.A).
3. Mix the sample for 60–120 minutes at room temperature, ensuring the beads remain in suspension. We recommend using a rotary mixer or Eppendorf vortex mixer capable of speeds of at least 1,100rpm.
4. Place tube on the magnetic stand for 10 seconds. Remove the supernatant and save for troubleshooting if desired.

Wash

5. Add 250 μ l of bind/wash buffer to the tube and mix for 5 minutes.
6. Place tube on the magnetic stand for 10 seconds.
7. Remove and discard the buffer. Repeat two more times for a total of 3 washes using binding/wash buffer.

Elute (see Section 5 for a choice of elution buffers)

8. Add 50 μ l of elution buffer to the beads in the tube.
9. Mix for 10 minutes at room temperature, ensuring the beads remain in suspension (see Step 3).
10. Place the tubes in the magnetic stand for 10 seconds. Remove eluted sample and transfer to a second microcentrifuge tube.

Neutralize (see Section 5 for a choice of neutralization buffers)

11. Add 5 μ l of neutralization buffer to each microcentrifuge tube containing eluted sample.
12. Eluted samples can be analyzed by downstream applications or can be stored at -20°C .

4. Troubleshooting

Symptoms	Causes and Comments
Low or no capture of biotinylated antibody	Magnetic beads settled during binding. Increase the mixing speed to maintain beads in suspension during incubation.
Low or no recovery of target antibody	Magnetic beads settled during binding. Increase the mixing speed to maintain beads in suspension during incubation. Check pH of the elution buffer. Using an elution buffer with a pH greater than 3 will significantly reduce antibody yield.
Results appear to be imprecise	Low amount of antibody in the sample. Increase sample volume. Increase elution volume, ensure proper mixing and centrifugation, and ensure careful liquid transfer after elution.

5. Composition of Buffers and Solutions

Bind/Wash Buffer (25mM Tris, 150mM NaCl [pH 7.5])

3.0g Tris

8.76g NaCl

Combine in 800ml of deionized water. Adjust pH to 7.5.

Elution Buffers (Choose one of these two elution buffers based on your downstream application.)

Elution Buffer for Mass Spec Analysis

0.1% TFA

Elution Buffer for Non-Mass Spec Applications (100mM glycine-HCl buffer [pH 2.7])

0.375g glycine

Dissolve in deionized water. Adjust pH to 2.7 with HCl. Bring to a final volume of 50ml with deionized water.

Neutralization Buffers (Choose one of these two neutralization buffers based on your downstream application.)

Neutralization Buffer for Mass Spec Analysis (500mM ammonium bicarbonate [pH 7.8])

3.95g ammonium bicarbonate

Add ammonium bicarbonate to 100ml of nanopure water. Adjust pH to 7.8.

Neutralization Buffer for Non-Mass Spec Applications (2M Tris buffer [pH 7.5])

0.472g Trizma base

2.54g Trizma hydrochloride

Dissolve in deionized water. Adjust pH to 7.5. Bring the final volume to 10ml with deionized water.



6. Related Products

PRODUCT	SIZE	CAT.#
Goat Anti-Human Biotinylated IgG	4ml	V7830
PolyATtract® System 1000 Magnetic Separation Stand	1 each	Z5410
MagneSphere® Technology Magnetic Separation Stand (two-position)	0.5ml	Z5331
	1.5ml	Z5332
	12 × 75mm	Z5333
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341
	1.5ml	Z5342
	12 × 75mm	Z5343

7. Summary of Changes

The following change was made at the 10/18 revision of this Technical Manual:

Table 1 was updated to correct the name of the product “Goat Anti-Human Biotinylated IgG”.

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