Sera-Mag™ SpeedBeads Streptavidin-Blocked Magnetic Particles

Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles (Table 1) use a non-surfactant, non-protein blocking reagent and provide low nonspecific binding (NSB), and high binding capacity for biotinylated target molecules.

Typical applications include:

- improvement and simplification of ligand binding
- affinity purifications
- immunoprecipitation
- protein interaction studies
- DNA: protein pulldowns
- purification of biotin-labeled proteins and nucleic acids
- other molecular biology applications

Biotinylated molecules are bound to the magnetic particles which are removed from the solution using a magnetic field. For manual processing, a simple magnetic stand can be used. An automated platform can also be used for magnetic purification.

Streptavidin is a M_r 60 000 protein from *Streptomycetes avidinii* (1). The protein is a tetramer containing four biotin-binding sites and is covalently coupled to the surface of blocked magnetic particles. There are two to three biotin binding sites available for each streptavidin molecule bound to the microparticle surface. Unlike avidin, streptavidin has a low isoelectric point (pI=5) and no carbohydrate groups, resulting in low non-specific binding. Furthermore, the protein is coupled to magnetic particles that exhibit very low non-specific binding in the presence of complex biological samples. The affinity between streptavidin and biotin is very high, requiring harsh conditions for disruption such as SDS-PAGE reducing sample buffer. Therefore, it is possible to elute binding partners in an interaction complex without co-eluting the biotinylated component.

Sera-Mag SpeedBeads combine the advantages of a high surface area, high affinity and high specific activity. They are colloidally stable in the absence of a magnetic field. However, the particles can be separated rapidly and completely from suspension when a magnetic field is applied. Binding of biotinylated ligands to streptavidin groups on the surface is easily accomplished using standard avidin-biotin technology.

In the past, achieving high activity and stable binding of solid phase ligands has been a major difficulty. Compounds that are difficult to attach to microparticle surfaces by conventional means may be amenable to biotinylation. Due to the high affinity of the avidin-biotin reaction, binding biotinylated compounds to Sera-Mag SpeedBeads may improve specific activity. In such cases, biotinylation may be carried out in aqueous or organic solvent. Then, the biotin derivative can be bound to Sera-Mag SpeedBeads simply by mixing in appropriate buffer conditions. For example, nucleic acids which adsorb poorly to microparticle surfaces are readily bound to Sera-Mag SpeedBeads particles after biotinylation.

The use of magnetic particles as a solid phase support in immunoassays and molecular biology applications is well documented. Standard protocols are available to biotinylate a wide range of ligands including proteins, nucleic acids, haptens, peptides, and other molecules

Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles are uniform, colloidally stable, monodispersed, non-porous super-paramagnetic spheres made by a proprietary core shell process. The core is a carboxylate-modified particle made by free radical emulsion polymerization of styrene and acid monomer. Magnetite (Fe_3O_4) is coated onto this core particle and then encapsulated with propriety polymers. Finally, the surface is blocked with a proprietary method to help prevent the nonspecific binding of proteins.

Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles are nominal 1 µm particles with highly active streptavidin covalently bound to the surface. They are supplied at approximately 1% solids (10 mg/ml) in 0.05 % sodium azide.

Table 1. Characteristics of Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles

Composition:	Streptavidin monolayer covalently coupled to bead surface.
Magnetization:	Superparamagnetic (no magnetic memory).
Mean diameter:	1 µm (nomimal)
Bead concentration:	10 mg/ml (bead weight/volume); 1% solids
Binding capacity: (per mg of bead)	~ 3500 pmol biotinylated fluorescein
Particle density:	~ 2.0 g/cm^3

Note : Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles are not supplied in RNase-free solutions

Important information before using Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles

- Do not freeze or dry Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles. This causes the particles to aggregate and lose binding activity.
- After labeling proteins or nucleic acids with biotin, remove unincorporated biotin with a desalting column. Free biotin will reduce the binding capacity of the particles.
- To minimize protein degradation, include protease inhibitors in the preparation of cell lysate.
- A low pH elution may be used for single-use applications. To limit leaching of streptavidin, do not exceed 10 min for the elution step in either manual or automated protocols.
- Boiling the magnetic particles in SDS-PAGE reducing sample buffer is acceptable for single-use applications. Boiling causes microparticle aggregation and loss of binding activity.
- Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles can be used successfully with mass spectrometry because the non-specific binding is very low.

Procedure for manual immuno-precipitation using a biotinylated antibody

Additional materials required

- Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles
- 1.5 ml microcentrifuge tubes
- Sera-Mag Buffer Kit (code number 281111)
  - Hydridization, wash and elution buffers included in kit.
- Alternate elution buffer: SDS-PAGE reducing sample buffer
- Biotinylated antibody
- Antigen sample
- Cell lysis buffer (used to prepare antigen sample)
- Magnetic stand for 1.5 ml tube (e.g., MagRack 6, GE Healthcare code number 28-9489-64)

Prewashing Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles

Note: To ensure homogeneity, mix the particles thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.

1. Aliquot 50 µl (0.5 mg) of Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles into a 1.5 ml microcentrifuge tube.

2. Place the tube into a magnetic stand to collect the particles against the side of the tube. Remove and discard the supernatant.

3. Add 1 ml of binding/wash buffer to the tube. Invert the tube several times or vortex gently to mix. Collect the particles with a magnetic stand, then remove and discard the supernatant.

Note : Do not allow the particles to dry. If necessary, store them in binding/wash buffer prior to proceeding with purification protocol.

Immunoprecipitation

Note: This protocol is a general guideline for immunoprecipitation and requires optimization for each application.

4. Combine antigen sample with 10 µg of biotinylated antibody. Incubate 1 to 2 h at room temperature or overnight at 4˚C with mixing.

Note: Dilute each sample to a minimum volume of 300 µl with cell lysis buffer or binding/wash buffer.

5. Add the antigen sample/biotinylated antibody mixture to a 1.5 ml microcentrifuge tube containing prewashed magnetic beads (see above) and incubate at room temperature for 1 h with mixing.

6. Collect the particles with a magnetic stand, remove the supernatant and save for analysis.

7. Add 300 µl of binding/wash buffer to the tube and gently mix. Collect the particles and then discard the supernatant. Repeat twice.

8. Elution buffer recovery of antigen: Add 100 µl of elution buffer to the tube. Incubate the tube at room temperature with mixing for 5 min. Magnetically separate the particles and save the supernatant containing target antigen.

Note: If a low pH elution buffer is selected for elution, streptavidin may leach from the particles. Low pH elution buffers are effective for most antibody-antigen interactions. However, to ensure efficient release of target antigen from the antibody, prerinse the particles with 300 µl 0.1% Tween™-20 in water (no buffering capacity) before adding low pH elution buffer.

Alternate Elution: SDS-PAGE reducing sample buffer recovery of antigen: Add 100 µl of SDS-PAGE reducing sample buffer to the tube and heat the samples at 96°C to 100°C in a heating block for 5 min. Magnetically separate the particles and save the supernatant containing the target antigen.

Note: If SDS-PAGE buffer is selected for elution, the eluate will contain streptavidin monomers and dimers and biotinylated antibody along with target antigen.

Procedure for automated immunoprecipitation with biotinylated antibody

Additional Materials Required

- KingFisher™ Flex with 96 deep well head (Thermo Scientific product number 5400630) or KingFisher 96 (Thermo Scientific product number 5400500)
- KingFisher Flex Microtiter Deepwell 96 plate, V-bottom (Thermo Scientific product number 95040450, 50 pcs)
- KingFisher 96 tip comb for Deep Well magnets (Thermo Scientific product number 97002534, 10 x 10 pcs/box)
- 1.5 ml microcentrifuge tubes
- Sera-Mag Buffer Kit (code number 281111)
  - Hydridization, wash and elution buffers included in kit.
- Alternate elution buffer: SDS-PAGE reducing sample buffer
- Antigen sample
- Biotinylated antibody

Preparation of KingFisher instrument and plate set-up

Note: The following protocol is designed for general use with the KingFisher Flex or KingFisher 96 Instrument. The protocol can be modified according to customer needs using the Thermo Scientific BindIt™ software provided with the instrument.

1. Combine antigen sample with 10 µg of biotinylated antibody per sample. Incubate 1 to 2 h at room temperature or overnight at 4˚C with mixing.

2. Download the "SA Immunoprecipitation low pH elution" or "SA Immunoprecipitation heated elution" protocol from www.thermoscientific.com/kingfisher into the BindIt software on an external computer.

3. Transfer the protocol to the KingFisher Flex or KingFisher 96 from an external computer. See the BindIt software user manual for detailed instructions on importing protocols.

4. Set up the plates according to Table 1 which will subsequently be placed into the KingFisher Flex or KingFisher 96 instrument.

Notes

- If using less than 96 wells, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- To ensure particle homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or rotating platform before adding the particles to plate 1.
- Combine the Tip Comb with a Deep Well 96 plate. See KingFisher Flex or KingFisher 96 user manual for detailed instructions.
- The particles can be eluted into 100 µl of 0.1 M glycine, pH 2-3 or 100 µl SDS-PAGE reducing sample buffer. If using SDS-PAGE reducing sample buffer in a heated elution, install the KingFisher Flex or 96 Heating Block (see manual for proper installation) to heat samples at 96°C to 100°C for 10 min.
- If you select SDS-PAGE reducing sample buffer for elution and will be performing a Western blot using rabbit antibodies (primary or secondary), do not heat the samples. Incubate at room temperature for 10 min.
- If low-pH elution buffer is selected for elution, neutralize the pH using 10 µl neutralization buffer for each 100 µl of eluate upon run completion.
- To limit evaporation, select "Mix" and "Slow" speed under the subheading "Heating Action".

Executing the SA Immunoprecipitation Protocol on the KingFisher 96

1. Select the protocol using the arrow keys in the instrument keypad and press Start. See the instrument user manual for detailed information.

2. Slide open the door of the protective cover of the instrument. Load the plates into the instrument according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing Start.

3. After sample processing, remove the plates as instructed by the instrument display. Press Start after removing each plate.

4. Press Stop after all plates are removed.

Table 1. Pipeting instructions for immunoprecipitation protocol

Plate #	Plate Name	Plate type	Content	
1	Beads	Microtiter Deep Well 96 Plate	Streptavidin beads	50 µl
2	Bead wash	Microtiter Deep Well 96 Plate	Binding/Wash buffer	150 µl
3	Antigen sample	Microtiter Deep Well 96 Plate	Binding/Wash buffer	1000 µl
4	Wash 1	Microtiter Deep Well 96 Plate	Antibody/ Antigen sample	300 µl
5	Wash 2	Microtiter Deep Well 96 Plate	Binding/Wash buffer	300 µl
6	Wash 3	Microtiter Deep Well 96 Plate	0.1% Tween-20 in water	300 µl
7	Elution (Immuno-precipitation low pH elution)	Microtiter Deep Well 96 Plate	Elution buffer	100 µl
7	Elution (Immuno-precipitation heated elution)	Microtiter Deep Well 96 Plate	SDS-PAGE reducing sample buffer	100 µl
8	Tip plate	Microtiter Deep Well 96 Plate	King Fisher Flex 96 tip comb for Deep Well magnets	-

General troubleshooting tips and suggestions for Sera-Mag SpeedBeads Streptavidin-Blocked Magnetic Particles

Problem	Possible Cause	Solution
Low protein recovery	Proteolysis of sample	Add protease inhibitors
	Not enough magnetic beads used for capture	Increase the amount of magnetic beads used for capture
	Insufficient target protein present in sample	Increase amount of antigen sample
	Free biotin present in sample	Dialyze biotinylated antibody or pass it through a desalting column prior to binding to the magnetic beads
Protein does not elute	Elution conditions are too mild	Increase incubation time with elution buffer or use more stringent elution buffer
Multiple, non-specific bands appear in eluted sample	Non-specific protein binding to the magnetic beads	Add 50-200 mM NaCl to the binding/wash and /or elution buffers
Magnetic beads aggregate	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions
	Buffer used is incompatible with magnetic beads	

References

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

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For local office contact information, visit:
www.gelifesciences.com/contact

www.gelifesciences.com/sera-mag

GE Healthcare UK Limited
Amersham Place
Little Chalfont,
Buckinghamshire, HP7 9NA,
UK

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1
Hyakunincho Shinjuku-ku
Tokyo 169-0073
Japan

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