Sera-Mag™ Streptavidin, SpeedBeads Streptavidin and **Sera-Mag™** SpeedBeads NeutrAvidin™-Coated Magnetic Particles

PROTEIN ENRICHMENT

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Sera-Mag™ Streptavidin, Sera-Mag™ SpeedBeads Streptavidin and Sera-Mag™ SpeedBeads NeutrAvidin™-Coated Magnetic Particles combine fast reaction kinetics and low, nonspecific binding for increased throughput, precision and solid phase support in a variety of immunoassay and molecular biology applications. These include sample preparation and assay development for genomics and proteomics applications.

- Low dissociation constant (K_d 10^{-15} M) to tightly bind ligands.
- Covalently bound to prevent leaching from the surface.
- Even coating of SA or NA protein for reliable results.
- Multiple biotin binding capacities available for optimizing assay development (Sera-Mag™ Streptavidin only).
- Stability in biological buffer systems optimizes reagent development.
- No carbohydrate reduces nonspecific binding caused by sugars (NeutrAvidin™ only).
- Fast movement through viscous solutions.

Nominally 1 micron in diameter, Sera-Mag™ magnetic beads are well-suited for the majority of biological applications, and due to their superparamagnetic properties, they are very easy to handle. A core polystyrene particle is coated with magnetite, which is then encapsulated using a proprietary polymer to provide a suitable surface which is the basis for all Sera-Mag™ beads. A second layer of magnetite is applied, followed by further encapsulation, to form Sera-Mag™ SpeedBeads, which generate a faster magnetic response for viscous substrates or where speed to magnet is required (Figs 1 and 2).

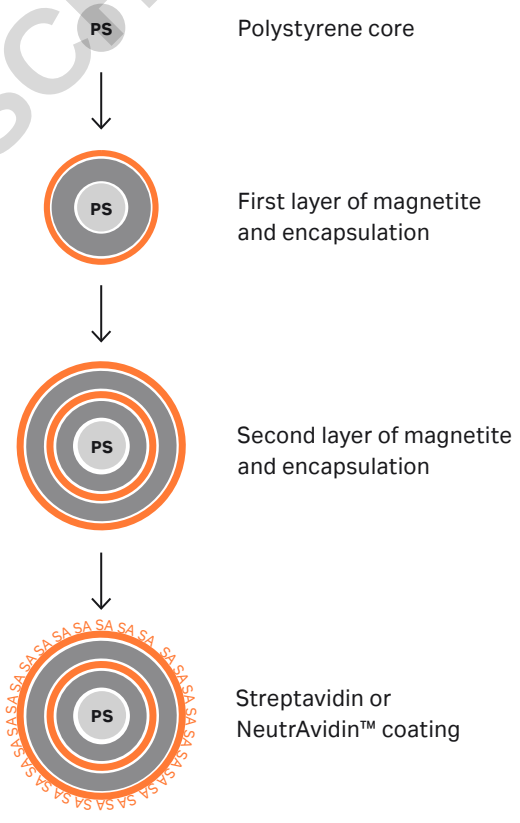


Fig 1. Sera-Mag™ SpeedBeads magnetic particles are made by a modified Sera-Mag™ process in which a larger quantity of magnetite is added as a second layer within the particle, resulting in a 2×-faster increase in speed in response to a magnetic field.

Compounds that are difficult to attach to particle surfaces by conventional means may be amenable to biotinylation. Streptavidin-Coated and NeutrAvidin™-Coated SpeedBeads are available with a 3500–4500 pmol/mg biotin-binding range.



Fig 2. Image developed from a scanning electron microscopy (SEM) showing the cauliflower-like surface of the Sera-Mag™ SpeedBeads magnetic particles. In addition to its high carboxylic acid content, the surface dramatically increases the overall surface area available for binding.

Streptavidin-coated and NeutrAvidin™-coated magnetic particles in molecular biology

Achieving high activity and stable binding of biological ligands to magnetic beads can often be technically challenging. Where direct conjugation to the bead surface is either unsuccessful or impractical, adoption of streptavidin-coated magnetic particles can provide a suitable and more convenient alternative where the ligand itself is biotinylated and captured on the bead surface. Standard protocols are available to biotinylate a wide range of ligands including proteins, nucleic acids, haptens, peptides, and other molecules.

Sera-Mag™ Streptavidin-Coated magnetic particles

The original Sera-Mag™ Streptavidin magnetic beads provide robust performance in a range of immunoassay and molecular biology techniques, exhibiting a high affinity when binding to biotinylated ligands such as proteins, nucleic acids and peptides. There are three different capacity ranges that provide flexibility to meet different needs (Table 1).

Sera-Mag™ SpeedBeads Streptavidin-Coated magnetic particles

Streptavidin-Coated SpeedBeads provide a high biotin-binding capacity along with a strong affinity for targeted, biotin-labeled molecules, providing an ideal solid phase support for immunoassays and molecular biology applications.

Sera-Mag™ SpeedBeads Streptavidin-Blocked Magnet Particles

Sera-Mag™ SpeedBeads Streptavidin-Blocked Magnetic Particles are designed for demanding applications where high binding capacity and low non-specific binding are essential to meet the required levels of performance. Unlike other blocked beads, Sera-Mag SpeedBeads Streptavidin-Blocked beads have a chemically coated surface that reduces the non-specific adsorption of non-targeted molecules. The coupling procedure provides a uniform monolayer of streptavidin molecules across the surface. The SpeedBeads format also provides fast magnetic response time adding convenience and ease of use.

Sera-Mag™ SpeedBeads NeutrAvidin™-Coated Magnetic Particles

Sera-Mag™ SpeedBeads NeutrAvidin™-Coated Magnetic Particles use an alternative ligand for the binding of biotinylated targets or baits. NeutrAvidin™, derived from avidin, retains the same binding characteristics as streptavidin, but is processed to remove oligosaccharides and to lower the isoelectric point to nearly neutral. As such, NeutrAvidin™ offers the lowest non-specific binding, especially with negatively charged cell surfaces and nucleic acids, making it well-suited for a wide array of applications where non-specific binding is critical.

Table 1. Sera-Mag™ magnetic beads come in a range of formats with different binding capacities, magnetic response times and surface chemistries

Product code	Description	Binding capacity (pmol biotin/mg bead)	Concentration (% bead solids w/w)
30152103010350	Sera-Mag™ Streptavidin-Coated	2333-3545	0.9-1-1
30152104010350	Sera-Mag™ Streptavidin-Coated	3546-4454	0.9-1-1
30152105010350	Sera-Mag™ Streptavidin-Coated	4455-6091	0.9-1-1
66152104010350	Sera-Mag™ Speedbeads Streptavidin-Coated	3431-5357	0.9-1-1
21152104010350	Sera-Mag™ Speedbeads Streptavidin-Blocked	2500-5000	0.9-1-1
78152104010350	Sera-Mag™ Speedbeads NeutrAvidin™-Coated	3000-4000	0.9-1-1

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Binding of biotinylated species

Both streptavidin and NeutrAvidin™ are characterized by their affinity for biotin and are capable of binding up to four biotins per molecule in free solution.

The binding of biotin to streptavidin and similar proteins has been used for assay development and enrichment or pulldown techniques for many years and takes advantage of key properties of this affinity:

- **Rapid**: Attainment of equilibrium association can occur within seconds, depending on conditions.
- **Strong**: The dissociation constant of the avidin-biotin complex, KD, is ~ 10−15 M, making it one of the strongest known non-covalent bonds. The binding is difficult to reverse, making it essentially permanent under most assay conditions. Consequently, interaction is mild making this an attractive alternative to covalent bonding for the construction of assay formats.

Sera-Mag™ Streptavidin beads are available in a range of formats (Table 1).

Methods for determining biotin binding capacity of streptavidin-coated Sera-Mag™ beads

For streptavidin-coated beads in general, biotin binding capacity is most commonly assessed by use of a biotinylated reporter.

Direct and accurate measurement of biotin binding is not practicable, and for this reason measurements are carried out by way of a reporter such as tritium, or more commonly by use of fluorescently labeled biotin, e.g. fluorescein-biotin (Fig 3).

Here, a defined mass of streptavidin-coated magnetic beads is mixed with a known concentration of fluorescein-biotin, allowed to bind to capacity and subsequently removed using a magnet. The concentration of the remaining solution is measured and the reduction due to binding can be calculated. The capacity is reported in picomoles of biotinylated species bound per milligram of beads (pmoL/mg).

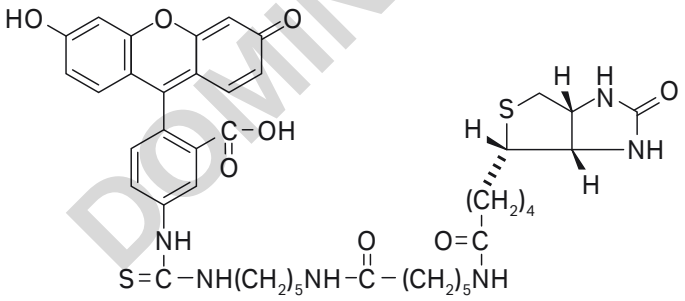


Fig 3. Fluorescein-biotin.

Binding of Sera-Mag™ Streptavidin beads to free biotin

An alternative approach is the use of 2-(4-hydroxyazobenzene) benzoic acid (HABA) to measure the binding capacity (Fig 4).

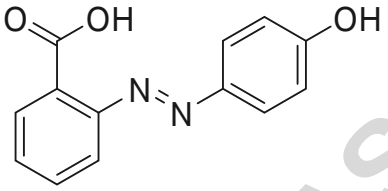


Fig 4. HABA 2-(4-hydroxyazobenzene) benzoic acid (HABA).

In this assay, the HABA dye binds to streptavidin on the bead surface in the same binding pocket as biotin, but with a weaker interaction. This allows displacement of the HABA when a solution of free biotin is added. This is accompanied by a measurable shift in visible absorbance of the dye, which is commonly detected at around 500 nm. HABA that is bound to avidin appears orange in colour (with a higher absorbance at 500 nm), while displaced or free HABA is a lighter yellow (lower absorbance at 500 nm).

These methods determine the capacity to bind a relatively small molecule, so are therefore a good indication of the number of accessible binding sites for a given mass of beads. However, this does not necessarily correlate with binding capacity in many applications. Larger molecules like antibodies, once bound, will obscure neighbouring sites and make them unavailable for interaction, so in these cases, only a small portion of possible binding sites will be occupied even at full binding capacity. This physical hindrance can also be seen where the antibodies may bind very close together, effectively blocking each other from binding their epitope. For this reason, biotin binding capacity is only considered an indicator of performance.

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Capture of BSA and IgG using Sera-Mag™ Streptavidin-Coated magnetic beads

To give an indication of binding capacity of Sera-Mag™ magnetic beads in biological applications, BSA (MWt 66.4KDa) and IgG (MWt 150KDa) were each biotinylated and captured to saturation using different Sera-Mag™ magnetic beads, and the values reported in Table 2.

Table 2. Testing results showed the broad range of binding capacities for current Sera-Mag™ beads for biotinylated IgG is around 40–80 µg IgG/mg bead

Sera-Mag™ bead type	Biotin-fluorescein binding (pmol/mg bead)	Biotin-BSA binding (µg/mg bead)	Biotin-IgG binding (µg/mg bead)
Sera-Mag™ Streptavidin 2500 to 3500 (Low) pmol per mg	2979	18.0	40.6
Sera-Mag™ Streptavidin 3500 to 4500 (Med.) pmol per mg	4030	28.2	58.9
Sera-Mag™ Streptavidin 4500 to 5500 (High) pmol per mg	5163	30.6	63.9
Sera-Mag™ Streptavidin 3500 to 4500 (Med.) pmol per mg	4261	36.5	77.7
Sera-Mag™ SpeedBeads Streptavidin-Blocked Magnetic Particles	3706	29.9	58.9

Effect of heating on biotin-streptavidin binding

Streptavidin-based magnetic particles are often heated during use and the biotin-streptavidin bond is considered irreversible in all but extreme circumstances. Like all proteins, streptavidin is subject to denaturation if heated to elevated temperatures, and once denatured, streptavidin is not effective at binding biotinylated species. As temperature increases above 70°C, binding capabilities begin to drop (Fig 5).

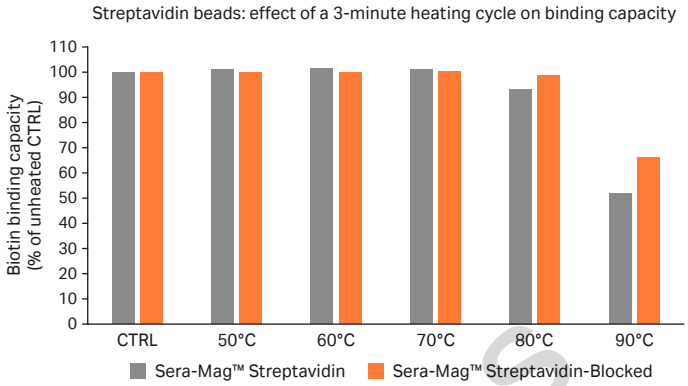


Fig 5. Binding capacities remain unaffected at temperatures up to 70°C, with significant reduction at 90°C.

However, where streptavidin is pre-bound with a ligand before heating, it has been shown to add stability: the interaction is able to withstand higher temperatures. Sera-Mag™ Streptavidin-Coated beads were bound to capacity with biotin and subjected to heating beyond the point of denaturation of native streptavidin to investigate retention of the binding capacity (Fig 6).

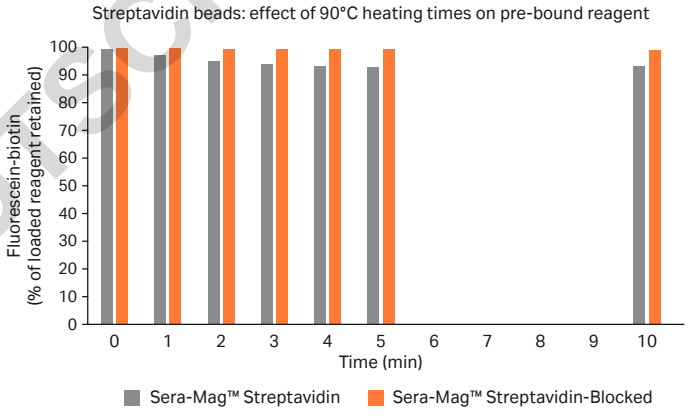


Fig 6. Compared to the significant reductions in binding capacity post-heating observed previously, the retention of pre-loaded ligand at the elevated temperature is much less affected. Only very low levels of fluorescein absorbance were detected in the post-heating supernatants, indicating >90% retention at 90°C for 10 minutes.

Use of streptavidin and other similar ligands to capture biotinylated targets or bait molecules is common in the biological sciences, from immunodiagnostic assay development to target enrichment in nucleic acid sequencing, each requiring slightly different characteristics. The range of Sera-Mag™ Streptavidin- and NeutrAvidin™-coated magnetic beads enables the user to select the most suitable bead for their chosen application to optimize results.

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Specifications

	Streptavidin	NeutrAvidin™
Particle composition		
Sera-Mag™ SpeedBeads	Double layer of magnetite	Double layer of magnetite
Sera-Mag™	Single layer of magnetite	N/A
Final encapsulation	Polymer with covalently conjugated streptavidin groups	Polymer with covalently conjugated NeutrAvidin™ groups
Particle density		
Sera-Mag™ SpeedBeads	~2.0 g/cm^3	~2.0 g/cm^3
Sera-Mag™	~1.7 g/cm^3	N/A
Magnetite content		
Sera-Mag™ SpeedBeads	~60%	~60%
Sera-Mag™	~40%	N/A
Protein surface		
Molecular weight	53 000	60 000
Number of binding sites	4	4
Isoelectric point (pH)	5.5	6.3
Biotin binding capacity (pmol/mg)		
Low	2500–3500	
Medium	3500–4500	3500–4500
High	4500–5500	
Product attributes		
Nominal diameter	1 µm	1 µm
pH stability	4 to 11	4 to 11
Additives	0.05% sodium azide	0.05% sodium azide
Storage conditions	Unless otherwise stated, refrigerate (2°C to 8°C) product when not in use, but do not freeze. Store upright and keep bottle tightly sealed. Mix product with gentle inversion by hand or vortex mixer.	

Ordering information

Product name	Pack size*	Product code
Sera-Mag™ Streptavidin-Coated magnetic particles (Low)	1 mL 5 mL 100 mL	30152103011150 30152103010150 30152103010350
Sera-Mag™ Streptavidin-Coated magnetic particles (Med.)	1 mL 5 mL 100 mL	30152104011150 30152104010150 30152104010350
Sera-Mag™ Streptavidin-Coated magnetic particles (High)	1 mL 5 mL 100 mL	30152105011150 30152105010150 30152105010350
Sera-Mag™ SpeedBeads Streptavidin-Coated magnetic particles (Med.)	1 mL 5 mL 100 mL	66152104011150 66152104010150 66152104010350
Sera-Mag™ SpeedBeads Streptavidin-Blocked Magnetic Particles	1 mL 5 mL 100 mL	21152104011150 21152104010150 21152104010350
Sera-Mag™ SpeedBeads NeutrAvidin™-Coated Magnetic Particles (Med.)	1 mL 5 mL 100 mL	78152104011150 78152104010150 78152104010350

*Larger volumes are available on request

Related products	Pack size	Product code
Sera-Mag™ SpeedBead Carboxylate-Modified [E7] Magnetic Particles	15 mL 100 mL 1000 mL	45152105050250 45152105050350 45152105050450
Sera-Mag™ SpeedBead Carboxylate-Modified [E3] Magnetic Particles	15 mL 100 mL 1000 mL	65152105050250 65152105050350 65152105050450
Sera-Mag™ Carboxylate-Modified [E7] Magnetic Particles	15 mL 100 mL 1000 mL	24152105050250 24152105050350 24152105050450
Sera-Mag™ Carboxylate-Modified [E3] Magnetic Particles	15 mL 100 mL 1000 mL	44152105050250 44152105050350 44152105050450
SeraSil-Mag™ 400	5 mL 60 mL 450 mL	29357369 29357371 29357372
SeraSil-Mag™ 700	5 mL 60 mL 450 mL	29357373 29357374 29357375
MagRack 6	1.5 mL / 2.0 mL microtubes	28948964

For more information on magnetic bead chemistries, handling properties and applications, visit our 'Scientist's Guide to Magnetic Beads'.

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