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

Instructions for Use

1 About this instruction

This instruction provides information for use of Sera-Mag™ and Sera-Mag SpeedBead Streptavidin-Coated, NeutrAvidin™-Coated, and Streptavidin-Blocked Magnetic Particles. These products can be used as a universal base particle for coating biotinylated proteins, oligos, or other ligands to the particle surface and providing solid phase support in immunoassays and molecular biology applications.

Sera-Mag and Sera-Mag SpeedBead Streptavidin-Coated, NeutrAvidin-Coated, and Streptavidin-Blocked Magnetic Particles are referred as "the particles" or "the beads" interchangeably in this instruction.

Find your local support representative at *cytiva.com/contact*.

2 Product codes

Product codes	Description	Pack Size
30152103011150	Sera-Mag Streptavidin-Coated Magnetic Particles 2500 to 3500 (low) pmol per mg	1 mL
30152103010150	Sera-Mag Streptavidin-Coated Magnetic Particles 2500 to 3500 (low) pmol per mg	5 mL
30152103010350	Sera-Mag Streptavidin-Coated Magnetic Particles 2500 to 3500 (low) pmol per mg	100 mL
30152104011150	Sera-Mag Streptavidin-Coated Magnetic Particles 3500 to 4500 (med) pmol per mg	1 mL
30152104010150	Sera-Mag Streptavidin-Coated Magnetic Particles 3500 to 4500 (med) pmol per mg	5 mL
30152104010350	Sera-Mag Streptavidin-Coated Magnetic Particles 3500 to 4500 (med) pmol per mg	100 mL
30152105011150	Sera-Mag Streptavidin-Coated Magnetic Particles 4500 to 5500 (high) pmol per mg	1 mL
30152105010150	Sera-Mag Streptavidin-Coated Magnetic Particles 4500 to 5500 (high) pmol per mg	5 mL
66152104011150	Sera-Mag SpeedBead Strepdavidin-Coated Magnetic Particles 3500 to 4500 (med) pmol per mg	1 mL
66152104010150	Sera-Mag SpeedBead Strepdavidin-Coated Magnetic Particles 3500 to 4500 (med) pmol per mg	5 mL
66152104010350	Sera-Mag SpeedBead Strepdavidin-Coated Magnetic Particles 3500 to 4500 (med) pmol per mg	100 mL
21152104011150	Sera-Mag SpeedBead Streptavidin-Blocked Magnetic Particles	1 mL
21152104010150	Sera-Mag SpeedBead Streptavidin-Blocked Magnetic Particles	5 mL
21152104010350	Sera-Mag SpeedBead Streptavidin-Blocked Magnetic Particles	100 mL
78152104011150	Sera-Mag SpeedBead NeutrAvidin-Coated Magnetic Particles	1 mL
78152104010150	Sera-Mag SpeedBead NeutrAvidin-Coated Magnetic Particles	5 mL
78152104010350	Sera-Mag SpeedBead NeutrAvidin-Coated Magnetic Particles	100 mL

3 Introduction

Important

Read the Instructions for Use carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

To handle the products in a safe way, refer to the Safety section in this instruction or to the Safety Data Sheets (where applicable).

CAUTION
Toxic if ingested. This product contains 0.05% sodium azide which is toxic if ingested. Do not pipette by mouth.

CAUTION
Explosive metal azides Sodium azide can react with lead and copper plumbing to form explosive metal azides. Dispose into a waste stream involving incineration.

4 Description

Overview

Sera-Mag and Sera-Mag SpeedBead Streptavidin-Coated, NeutrAvidin-Coated, and Streptavidin-Blocked Magnetic Particles are uniform, colloidally stable, monodispersed, non-porous particles made by a proprietary core-shell method covered with biotin-binding proteins.

Particle characteristics

The core of Sera-Mag Streptavidin-Coated Magnetic Particles is a carboxylate-modified particle made by free radical emulsion polymerization of styrene and acrylic acid. A single layer of magnetite (Fe_3O_4) is coated onto this core particle and then encapsulated with proprietary polymers.

The core of the Sera-Mag SpeedBead Streptavidin/NeutrAvidin-Coated, and Streptavidin-Blocked Magnetic Particles is made by a free radical emulsion polymerization of styrene and acid monomer. Two layers of magnetite are coated onto this core, resulting in fast magnetic response times (about 2x faster then Sera-Mag Streptavidin-Coated Magnetic Particles).

Surface characteristics

The particles are coated with proteins of the avidin class (streptavidin or NeutrAvidin) that bind biotin with one of the strongest non-covalent bonds known in nature. The binding interaction is effective over a wide range of chemical conditions (pH, levels of detergents, denaturants, and organic solvents). The surface is then blocked with a non-surfactant, non-protein-based method, to help prevent non-specific binding of proteins.

Contact your Cytiva support team for more information on other custom bead manufacturing methods.

Surface specificity: types of avidins

Sera-Mag Streptavidin-Coated Magnetic Particles and Sera-Mag SpeedBead Strepdavidin-Coated Magnetic Particles feature highly active streptavidin covalently bound to the surface, while Sera-Mag SpeedBead NeutrAvidin-Coated Magnetic Particles feature NeutrAvidin covalently bound to the surface. NeutrAvidin protein offers the advantages of a near-neutral isoelectric point (6.3). NeutrAvidin is deglycosylated and so does not have the RYD recognition sequence like streptavidin which means it has generally lower nonspecific binding than avidin and streptavidin and retains highest specific activity for biotin-binding proteins.

Surface specificity: organization

For Sera-Mag Streptavidin-Coated Magnetic Particles and Sera-Mag SpeedBead Streptavidin/NeutrAvidin-Coated Magnetic Particles, the avidin is covalently attached directly to the underlying carboxyl bead surface using EDAC-based chemistry. A one-step conjugation process attaches the avidin to the bead in a multilayer fashion i.e., there are avidin-avidin links as well as particle-avidin links. This gives the most efficient loading of the applied avidin onto the bead.

For Sera-Mag SpeedBead Streptavidin-Blocked Magnetic Particles, the avidin is covalently attached to the Sera-Mag SpeedBead Amine-Blocked Magnetic Particles, using glutaraldehyde linker chemistry. The conjugation process attaches the avidin to the bead in a monolayer fashion i.e., there are particle-avidin links but no avidin-avidin links. This gives a more organized surface and more efficient utilization of the avidin binding sites compared to other avidin coated beads. The underlying bead surface between the bound avidins retains the resistance to protein nonspecific binding associated with the Sera-Mag SpeedBead Amine-Blocked Magnetic Particles.

Specifications

All particles are produced internally using our proprietary manufacturing processes under strict Quality Systems Regulation practices.

Magnetization:	Superparamagnetic (no magnetic memory)
Mean diameter:	1 µm (nominal)
Bead concentration:	10 mg/mL (bead weight/volume); 1% solids
Bind capacity: (per mg of bead)	2500 to 5500 pmol biotinylated fluorescein (depending on bead type)[1]
Particle density:	about 2.0 g/cm^3

[1] Lot specific binding capacity bead specification can be found in the Certificate of Analysis (CofA) available for download at *https://www.cytivalifesciences.com/en/us/support/quality/certificates*.

The particles are not supplied in RNase-free solutions.

5 Typical Applications

Introduction

The particles can be used to link active biomolecules to a solid surface, in order to take advantage of their own specific activities and binding properties in downstream assays. The particles combine fast reaction kinetics and low nonspecific binding, and provide solid phase support for increased throughput and precision in a variety of immunoassay and molecular biology applications. Moreover, compounds that are difficult to attach to particles surfaces by conventional means may be amenable to biotinylation and then bound to biotin-binding particles.

The particles can be used for many molecular biology application including:

- protein-protein interactions
- protein-DNA interactions
- sample preparation and assay development for genomics and proteomics applications
- automated immunoassays
- immunoprecipitation
- purification of labeled proteins and nucleic acids

The particles can also be used successfully with mass spectrometry because the non-specific binding is very low.

Visit our application library *https://www.cytivalifesciences.com/en/us/solutions/genomics* for a list of published articles referencing the Sera-Mag and Sera-Mag SpeedBead Streptavidin-Coated, NeutrAvidin-Coated, and Streptavidin-Blocked Magnetic Particles.

Applications based on type of avidin

Streptavidin-coated magnetic particles provide a high biotin-binding capacity and a strong affinity for targeted, biotin-labeled molecules. Streptavidin-coated magnetic particles can be used in applications where the particles may be subjected to elevated temperatures e.g., when subjected to thermal cycling temperatures during PCR.

NeutrAvidin-coated magnetic particles are well-suited for a wide array of applications where nonspecific binding is critical. NeutrAvidin has no carbohydrates and a virtually neutral isoelectric point, making it less prone to nonspecific binding compared to streptavidin.

Application based on different binding capacity

Sera-Mag Streptavidin-Coated Magnetic Particles are available in low, medium, and high biotin binding capacities. High binding capacity particles can be used to bind an applied biotinylated compound using a lower mass of beads to minimize any unwanted bead interactions. The increased capacity of binding allow applications where high concentration of beads in suspension is not practicable.

However, increasing biomolecule binding by using a particle with a higher nominal biotin binding capacity may not be always effective due to steric constraints imposed by the biotinylated biomolecule. This is particularly true for larger biotinylated biomolecules, such as biotinylated IgG.

6 Handling

Introduction

Effective processing is one of the most critical aspects when using magnetic particles. Monodispersity and homogeneous suspension of particles should be carefully controlled during use to provide robust and reproducible performance.

Follow the guidelines in the next sections to handle the particles effectively.

6.1 Storage and stability

Store the particles at 2°C to 8°C.

NOTICE
Do not freeze.

If particles have settled, resuspend by swirling, rolling, shaking, or sonicating. Particles will usually remain active and monodisperse when maintained at 2°C to 8°C.

Freezing the particles is not recommended. Even brief freezing can negatively impact the performance of the beads and cause:

- Irreversible aggregation, leading to reduced suspension stability (faster gravity settling) and reduced binding capacity for biotinylated species.
- Disruption of bead structure, leading to leaching/release of component chemicals e.g., iron salts, and separation of the outer magnetite layers from the polystyrene core particle.

The particles are stable in a variety of molecular biology conditions such as guanidinium lysis buffers and PCR thermal cycling temperatures.

Note: *Drying of particles around the rim of the container can occur during handling and processing. If dried particle residue falls into the container, it can be easily removed by filtration.*

6.2 Resuspension

Incomplete particle resuspension might cause assay development issues.

Sonicate to resuspend the particles. Use a probe type ultrasonicator to resuspend particles after long term storage and centrifugation washing steps to reverse mild aggregation induced by coupling reactions.

6.3 Mixing

Procedure

When handling particles, mix the material to make sure that is monodispersed and uniformly distributed.

Step	Action
1	Select a suitable mixing option for your application from the table below to mix the particles.
2	Observe the particles during mixing to make sure that there is adequate agitation. If excessive foaming is observed, reduce the speed and time of mixing to minimize damage to the particles.
3	Confirm that the particles are adequately mixed. • Make sure that no product remains settled on the bottom of the container, especially after resuspension or • Confirm the absence of aggregates by observing the mixed particles under a microscope at 400x magnification.

Mixing options

Mixer	Application	Volume considerations	Recommended speed/time	Tips
Roller mixer	Resuspend and mix in a closed container	Containers must be 50% to 90% full to prevent insufficient mixing.	Minimum time: Vol < 1 L: 40 min Vol ≥ 1 L: 60 min Maximum time: 72 h	Containers with small diameter reduce mixing time. High particle concentrations increase mixing time.
Vortex mixer	Resuspend and mix in small containers (≤ 15 mL)	Up to 10 mL	80% of full speed	If resuspending mix for ≥ 1 min

Mixer	Application	Volume considerations	Recommended speed/time	Tips
Overhead mixer	Pooling, diluting, and handling large batches	Use a container so that the agitator blade is submerged.	Speed: increase the speed until movement is seen. Minimum time: Vol < 1 L: 40 min Vol < 1 L: 60 min	Place the agitator blade in the lower one-third of the container Avoid splashing on the side of the container

6.4 Sonication

Overview

The particles can be briefly sonicated using a probe sonicator to minimize clumping or aggregation. An immersible ultrasonic probe is the preferred method for efficient resuspension of particle pellets. Vortex mixing and bath-type sonicators can also be used but may not be effective enough for resuspending most particle pellets.

Do not sonicate volumes < 10 mL to avoid damaging particles. Sonicating small sample volumes heats the solution quickly because the volume is too low to sufficiently disperse the heat. Use vortex mixing instead of sonication.

Materials required for sonication

- Probe sonicator
- Sonicator probe: tapered microtip (1/8 inch diameter) or large tip probe (1/2 inch diameter)
- Container for sonication
- Optical microscope with 400× magnification.

Sonication protocol with a probe sonicator

Follow the steps below to sonicate the particles with a probe sonicator.

Step	Action
1	Mix the beads before starting the sonication. Follow the recommended mixing procedure presented in the previous section.

Step	Action
2	Select the appropriate sonication parameters as shown in the table below.

Probe	Volume to be sonicated	Sonication time	Intensity
Microtip (1/8 inch)	10–50 mL 50–100 mL 100–1000 mL	30–40 s 30–45 s > 60–90 s	30% to 40% of the max intensity
Large (1/2 inch)	1 L 3 L > 3 L	5 min 30–45 s > 60–90 s	50% of the max intensity

Note:
If material in samples > 100 mL settles out of solution too quickly, mix it during sonication or sonicate using more repetitions of shorter times.

Note:
If sonicating in a 1 liter bottle, roll the bottle for 5 min after every period of sonication.

3	Mix the sample thoroughly and observe a portion under a microscope at 400× magnification. If aggregates are visible, repeat sonication and observation until clumps are not visible.

7 Modes of operation

Overview

Sera-Mag and Sera-Mag SpeedBead Streptavidin-Coated, NeutrAvidin-Coated, and Streptavidin-Blocked Magnetic Particles are universal base particle for coating biotinylated proteins, oligos or other ligands to the particle surface. The particles can be then used as solid phase support in immunoassays and molecular biology applications.

Follow the procedure in this chapter to bind biotinylated ligands to the particles.

General tips for protocols

Follow the suggestions below for the intended application.

- For binding biotinylated compounds, use buffers with a pH above 7.0 (phosphate, Tris, MOPSO, and similar buffers). Use a salt concentration between 100 to150 mM.

- Use an excess of biotinylated ligand to prevent crosslinking through multiple biotin-streptavidin bonds.
- Where ligand is limited, titer with free biotin to prevent crosslinking.
- 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.
- 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 particle aggregation and loss of binding activity.
- In the preparation of cell lysate, include protease inhibitors to minimize protein degradation.
- To reduce non-specific binding (NSB) for Sera-Mag Streptavidin-Coated Magnetic Particles and Sera-Mag SpeedBead Strepdavidin-Coated Magnetic Particles, change the stringency of the binding and washing buffers, or use Sera-Mag SpeedBead Streptavidin-Blocked Magnetic Particles, as these particles have an underlying surface that has been chemically modified to be more resistant to NSB.
- NeutrAvidin yields the lowest nonspecific binding among the known biotin binding proteins. To reduce NSB for Sera-Mag SpeedBead NeutrAvidin-Coated Magnetic Particles, adjust the stringency of the binding and washing buffers, use Sera-Mag SpeedBead Streptavidin-Blocked Magnetic Particles or add a blocking agent.

Binding of biotinylated compounds

Follow the steps below to bind biotinylated ligands to Sera-Mag and Sera-Mag SpeedBead Streptavidin-Coated, NeutrAvidin-Coated, and Streptavidin-Blocked Magnetic Particles

Step	Action
1	Mix the particles and the biotinylated ligand.
2	Incubate for 30 to 60 minutes with continued agitation (e.g., hula mixer, thermomixer, or rollers) to maintain a homogeneous suspension.
3	Wash with buffer using centrifugation, magnetic separation or tangential flow filtration to remove unbound ligands.
4	Use probe sonication to resuspend particles after centrifugation.

The particles are coated with the biotinylated ligand.

Note: *The biotin-streptavidin binding interaction might be broken reversibly by one of the following methods:*

- *incubation with excess free biotin*
- *incubation at elevated temperatures in pure water*

8 Troubleshooting

Binding of biotinylated species to avidin-coated particles

Problem	Possible cause	Corrective action
Insufficient biotinylated species bound to beads: low or only partial uptake of applied biotinylated species	Insufficient particles used	Run titration experiments to determine loading capacity of particles for intended target. Adjust particle amount accordingly.
	Applied species not completely biotinylated	Check biotinylation level of biomolecule for example via HABA-avidin test kit. Perform biotinylation of biomolecule with increased amount reactive biotin species, to make sure that a sufficient fraction of the biomolecule sample is biotinylated.
	Applied species contains free biotin e.g., from incompletely-purified biotinylation reaction. The free biotin competes with biotinylated biomolecule for binding to avidin-coated particles.	Purify biotinylated species to remove unbound biotin reagents, for example via dialysis or desalting column.

Binding of a target species to a particle coated with a biotinylated capture molecule (e.g., biotinylated antibody)

Problem	Possible cause	Corrective action
Low recovery of antigen	Antigen not captured: capture molecule has degraded	Add protease inhibitors.
	Elution conditions are too mild	Increase incubation time with elution buffer or use more stringent elution buffer.
Low recovery of antigen (specific for processes involving heating particles higher then 70°C)	In-process bead aggregation due to avidin denaturation	Avidin stability to heat denaturation improves with increasing biotinylation. Make sure that the avidins are efficiently utilized with biotinylated species or add free biotin to occupy unused binding sites.
Multiple, non-specific bands appear in eluted sample	Non-specific protein binding to the magnetic beads	Add 50 to 200 mM NaCl to the binding, washing, or elution buffers.

9 Related products

Product name	Product code
MagRack 6	28948964
MagRack Maxi	28986441

cytiva.com/Sera-Mag

For local office contact information, visit cytiva.com/contact

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