

Sera-Mag™ SpeedBead Amine Blocked Magnetic Particles

Instructions for Use

1 About this instruction

This instruction provides information for use of Sera-Mag™ SpeedBead Amine Blocked Magnetic Particles as base particles for covalent coupling of biomolecules such as proteins, peptides, and carbohydrates to amine groups on the bead surface. Once coated, the particles can be used for further applications like enzymatic reactions, protein purification, and cell separation.

Sera-Mag SpeedBead Amine Blocked Magnetic Particles are referred as "the particles" or "the beads" interchangeably in this instruction.

Find your local support representative at [cytiva.com/contact](https://www.cytiva.com/contact).

2 Product codes

Product codes	Description	Pack Size
19152104011150	Sera-Mag SpeedBead Amine Blocked Magnetic Particles	1 mL
19152104010150	Sera-Mag SpeedBead Amine Blocked Magnetic Particles	5 mL
19152104010350	Sera-Mag SpeedBead Amine Blocked 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 SpeedBead Amine Blocked Magnetic Particles are magnetic beads supplied as aqueous suspension of magnetic particles coated to provide free amine groups. They are ready to use for covalent coupling with proteins or other ligand and can be used in the magnetic separation and isolation of antibodies, proteins, cells, or bacteria from serum, cell culture supernatants, ascites, or other sample types.

Particle characteristics

Sera-Mag SpeedBead Amine Blocked Magnetic Particles are uniform, colloiddally stable, mono-dispersed, non-porous super-paramagnetic spheres made by a proprietary core-shell method. 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.

Surface characteristics

Sera-Mag SpeedBead Amine Blocked Magnetic Particles are not blocked with conventional physical blockers for example, BSA or surfactant but the surface of the particles is modified using a proprietary chemical method. This provides the source of amino groups for onward chemical conjugations and a blocking modification that reduces the nonspecific binding of unwanted proteins to the underlying bead surface.

Conjugation is normally accomplished via use of a dialdehyde linker (normally glutaraldehyde) in a multi-step process as described in this user guide.

Specifications

Sera-Mag SpeedBead Amine Blocked Magnetic Particles are supplied as a suspension of particles in water with concentration 1% w/w (approximately 10 mg/mL). The formulation also contains 0.05% w/w sodium azide as preservative.

Lot specific bead specification can be found in the Certificate of Analysis (CofA) available for download at <https://www.cytivalifesciences.com/en/us/support/quality/certificates>.

5 Typical applications

Sera-Mag SpeedBead Amine Blocked Magnetic Particles surface is covered with amine groups ($-NH_2$) that can be used for the immobilization of amine-reactive ligands such as proteins, peptides, carbohydrates, enzymes, and antibodies.

Sera-Mag SpeedBead Amine Blocked Magnetic Particles enable to perform multiple molecular biology application including:

- protein purification
- positive separation
- cell separation

Visit our application library <https://www.cytivalifesciences.com/en/us/solutions/genomics> for a list of published articles referencing the Sera-Mag SpeedBead Amine Blocked Magnetic Particles.

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.

Storage and stability

Store Sera-Mag SpeedBead Amine Blocked Magnetic 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.

Sera-Mag SpeedBead Amine Blocked Magnetic Particles are stable in proteomics applications such as protein-protein interactions, protein-DNA interactions, immunoassays, and purification of labeled proteins and nucleic acids.

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.*

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.

7 Protein conjugation

About this procedure

The following procedure describes the coupling of Sera-Mag SpeedBead Amine Blocked Magnetic Particles with a protein of choice via glutaraldehyde conjugation.

The aldehyde groups of the linker at one end first form imine bonds with the particle amino groups, then with the coating protein amino groups at the other end. Residual aldehyde groups are reacted away similarly with an amino group-bearing quencher (glycine) to prevent continuing unwanted conjugations downstream. Before final washing and formulation, the hydrolytically-unstable imine groups are reduced to stable secondary amino groups by reduction with cyanoborohydride.

The protocol is optimized for 10 mg of particles i.e., 1 mL of formulation suspension.

Additional materials required

- Coupling reagents:
 - Buffer A: 10 mM Pyridine, pH 6.0
 - Buffer B: 10 mM Pyridine, pH 6.0 or 50 mM Bicarbonate buffer, pH 10.0
 - Buffer C: 0.1 M Ethanolamine, pH 8.0 or 0.1 M Glycine, pH 8.0
 - Sodium cyanoborohydride
 - Glutaraldehyde
- Storage solution (any buffer of choice)
- Stock solution of the protein of choice

Note: *If possible, exchange protein stock solution into water only or into buffer B before conjugation.*

General tips for protocols

- Coupling efficiency and particle performance is protein specific and should be optimized to produce best results.
- Optimize the coupling buffers by choosing Buffer A, one of the two options of Buffer B, and one of the two options of Buffer C.
- Sonicate the particles during/after mixing to achieve a uniform suspension.
- After the procedure, test for the efficiency of the coupling process by performing a quantitation of attached protein on the particles (e.g., by micro-BCA assay) or by evaluation of resulting binding capacity (e.g., for streptavidin coating, via uptake of biotinylated fluorescein, or equivalent process for other proteins).

Pre-reaction wash

Follow the steps below to wash your beads before the reaction.

Step	Action
1	Place desired amount of particles into a 2 mL microcentrifuge tube.
2	Magnetically separate and aspirate the cleared storage solution to waste.
3	Wash the particles two times with Buffer A (1.0 mL each wash). For each wash: a. Add the buffer. b. Vortex for 15 seconds or a time sufficient to resuspend the pellet. c. Separate the particle using a magnetic stand. d. Aspire the cleared supernatant to waste.

Particle Activation

Follow the steps below activate Sera-Mag SpeedBead Amine Blocked Magnetic Particles with glutaraldehyde.

Step	Action
1	Resuspend particles to 2.5% solids with Buffer A (400 µl).
2	Add 100 µl of 25% glutaraldehyde solution per mg of particle. Note: <i>Final concentration during activation should have particle concentration to 2% solids, and glutaraldehyde concentration to 5%.</i>
3	Mix (roll or mechanically mix e.g., via thermomixer) for 3 hours at room temperature or at elevated temperatures up to 43°C.
4	Magnetically separate and aspirate the activation reaction solution to waste.
5	Wash particles three times with Buffer B (1.0 ml each wash). For each wash: a. Add the buffer. b. Vortex for 15 seconds or a time sufficient to resuspend the pellet. c. Separate the particle using a magnetic stand. d. Aspire the cleared supernatant to waste. <i>Result:</i> The particle are activated to the aldehyde blocked intermediate and ready to be coupled with the protein of choice. Proceed immediately to the coating of the activated particle.

Coating of activated particles

Follow the steps below to coat the activated particles with the protein of choice.

Step	Action
1	Resuspend final pellet to 4% solids with Buffer B (250 µL).
2	Dilute the protein stock solution in Buffer B such that the desired application mass of protein is contained within 250 µL. Note: <i>A typical application requires a ratio of protein to particles between 20 to 200 µg protein/mg particle.</i>

Step	Action
	Note: For example, for an applied ratio of protein to particles of 50 µg protein/mg particle, coating 10 mg of particles requires 500 µg of protein. For this to be contained in 250 µL of Buffer B, protein concentration should be 2.0 mg/mL. Note: If the protein stock solution contains a buffer different from water or Buffer B, make sure that the final concentration of this buffer after dilution is < 10mM.
3	Add the prepared protein solution in Buffer B (250 µL) to the particle suspension in Buffer B.
4	Mix (roll or mechanically mix e.g., via thermomixer) for 16 to 24 hours at room temperature or at elevated temperatures up to 43°C (depending on protein stability).

Quenching of the activation reaction

Follow the steps below to quench the unconjugated aldehyde groups of the particles.

Step	Action
1	Add 500 µL of Buffer C.
2	Mix (roll or mechanically mix e.g., via thermomixer) for 1 hour at room temperature.
3	Magnetically separate and aspirate the quenching reaction solution to waste.

Hydrolytic stability reaction

Follow the steps below to reduce the imine linkage bonds to secondary amines for hydrolytic stability of the particles.

Step	Action
1	Resuspend pellet to 2% solids with 500 µL Buffer B.
2	Prepare a 2% sodium cyanoborohydride stock solution in Buffer B. Use immediately. Do not store.
3	Add 500 µL sodium cyanoborohydride stock solution to a final concentration of 1% particle solid and 1% sodium cyanoborohydride.

Step	Action
4	Mix (roll or mechanically mix e.g., via thermomixer) for 1 hour at ambient temperature.
5	Magnetically separate and aspirate the reaction solution to waste.
	Note: <i>Treat as toxic cyanide waste.</i>

Particle washing and final formulation.

Follow the steps below to prepare the particles for storage.

Step	Action
1	Wash 3 to 6 times with storage solution (1 mL each wash). For each wash: - Add the buffer. - Vortex for 15 seconds or a time sufficient to resuspend the pellet. - Separate the particle using a magnetic stand. - Aspire the cleared supernatant to waste. Tip: <i>Include detergent in the storage buffer e.g., Tween™ 20 at 0.1% w/v to reduce bead aggregation, to prevent sticking to plasticware, and to remove non-covalently adsorbed protein.</i> Tip: <i>Additional washes with high salt concentration (1 M NaCl), mildly acidic, or basic media can be used to reduce non-covalently adsorbed protein. Other methods can include mildly elevated temperatures or increased exposure time during washes.</i>
2	Resuspend to final desired particle concentration with storage solution. Note: <i>Protein coated particles can undergo settling with prolonged storage. The product should be shaken vigorously before use. Do not freeze or dry. Particles coated with protein will benefit from storage in a saline buffer with added detergent e.g., Tween 20 at 0.1% w/v.</i>

Measure coupling capacity

Protein coupling efficiency can be determined by measuring the change in absorbance of the supernatant before and after coupling. This depends on the size of ligand coupled. Follow the steps below to measure the coupling capacity of the protein to Sera-Mag SpeedBead Amine Blocked Magnetic Particles.

Step	Action
1	Set spectrophotometer wavelength to 280nm.
2	Use Buffer B as blank.
3	Measure the absorbance at 280 nm of the Pre-Coupling Solution. (Abs _{pre}) Note: <i>Depending on the amount of protein added, a dilution may be necessary to read an absorbance. (D = dilution factor)</i>
4	Measure the absorbance at 280 nm of the Post-Coupling Solution. (Abs _{post}) Note: <i>A dilution may be necessary to read the absorbance (d = dilution factor)</i>
5	Calculate the coupling efficiency, expressed as the % Protein Uptake using the formula below:

$$\% \text{ Protein Uptake} = \frac{((\text{Abs}_{\text{pre}} \times D) - (\text{Abs}_{\text{post}} \times d))}{(\text{Abs}_{\text{pre}} \times D)} \times 100$$

8 Troubleshooting

Prior to use

Problem	Possible cause	Corrective action
Clumping prior to use	Settling during long-term storage	Mix carefully to make sure that the bead suspension is homogeneous e.g., by placing on rollers overnight. For speed of mixing, use pipette mixing or vortexing for small sample. Sonication can also be used but avoid high sonic power and long sonication times.

During conjugation

Problem	Possible cause	Corrective action
Clumping during coupling reaction	Glutaraldehyde addition	• Agitate beads. • Decrease bead concentration • Add an excess of glutaraldehyde to avoid chemical crosslinking of particles Clumping typically resolve by the conclusion of protein coupling.
	Protein addition	Increase protein concentration.
	Washing	Add surfactant or reduce number of washing steps.
Low or variable coating	Suboptimal coupling condition due to reagents	Use fresh molecular biology reagents.
	Suboptimal coupling condition due to protein	Make sure there are sufficient available amino groups on protein. Adjust the pH of buffer for protein coating closer to protein pI value
	Suboptimal coupling condition. Incomplete reduction of imine bonds: loss of coupled protein via imine link hydrolysis	Use a fresh unit of sodium cyanoborohydride to make sure that unstable imine groups are completely reduced to stable secondary amine.
Bead smearing on the tube wall	A sign of stickiness/hydrophobicity, which can cause particles to resist sedimentation	Add surfactant e.g., 0.001% to 0.1% Tween to wash/storage buffer. Use low protein binding microcentrifuge tubes.

9 Related products

Product name	Product code
MagRack 6	28948964
MagRack Maxi	28986441



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29513473 AA V:1 01/2022