Sera-Mag™ and **Sera-Mag™** SpeedBead Carboxylate-Modified Magnetic Particles

Instructions for Use

1 About this instruction

This instruction provides information for use of Sera-Mag™ and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles as a base particle for covalent coupling of biomolecules such as proteins and nucleic acids to carboxyl groups on the surface. Once coated, the particles can be used for further applications like protein purification and cell separation.

Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles are referred to 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
24152105050250	Sera-Mag Carboxylate-Modified [E7] Magnetic Particles	15 mL
24152105050350	Sera-Mag Carboxylate-Modified [E7] Magnetic Particles	100 mL
24152105050450	Sera-Mag Carboxylate-Modified [E7] Magnetic Particles	1000 mL
44152105050250	Sera-Mag Carboxylate-Modified [E3] Magnetic Particles	15 mL
44152105050350	Sera-Mag Carboxylate-Modified [E3] Magnetic Particles	100 mL
44152105050450	Sera-Mag Carboxylate-Modified [E3] Magnetic Particles	1000 mL
45152105050250	Sera-Mag SpeedBead Carboxylate-Modified [E7] Magnetic Particles	15 mL
45152105050350	Sera-Mag SpeedBead Carboxylate-Modified [E7] Magnetic Particles	100 mL
65152105050250	Sera-Mag SpeedBead Carboxylate-Modified [E3] Magnetic Particles	15 mL
65152105050350	Sera-Mag SpeedBead Carboxylate-Modified [E3] Magnetic Particles	100 mL
65152105050450	Sera-Mag SpeedBead Carboxylate-Modified [E3] Magnetic Particles	1000 mL

For simplification reasons:

- Sera-Mag Carboxylate-Modified [E7] Magnetic Particles and Sera-Mag Carboxylate-Modified [E3] Magnetic Particles are referred to, from this point on, as Sera-Mag Carboxylate-Modified Magnetic Particles, only.
- Sera-Mag SpeedBead Carboxylate-Modified [E7] Magnetic Particles and Sera-Mag SpeedBead Carboxylate-Modified [E3] Magnetic Particles are referred to, from this point on, as Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles, only.

For more information, see *cytiva.com/shop/molecular-biology/sequencing/magnetic-beads/sera-mag-speedbeads-and-sera-mag-carboxylate-modified-magnetic-particles-p-05936*, or contact your local Cytiva representative.

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.

4 Safety precautions

To handle the products in a safe way, refer to the Safety precautions 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.

5 Description

Overview

Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles are nominal 1 µm magnetic particles of uniform size with carboxylic groups on the surface. They combine a fast magnetic response time and high binding capacity with a large surface area, high sensitivity, stability, physical integrity, and fast reaction kinetics.

Particle characteristics

Sera-Mag Carboxylate-Modified Magnetic Particles and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles differ in the amount of magnetite (Fe_3O_4) content. Sera-Mag Carboxylate-Modified Magnetic Particles have a single magnetite layer (~ 40%) while Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles have a double layer of magnetite (~ 60%) and so responds about two times faster to magnetic fields.

Surface characteristics

The particles feature carboxylic groups on the surface that allow easy covalent coupling of primary amino groups of nucleic acids, peptides, proteins or other target molecules using simple carbodiimide chemistry.

Specifications

Lot specific bead specification can be found in the Certificate of Analysis (CofA) available for download at *cytiva.com/support/quality/certificates*.

6 Typical Applications

Covalent coupling of biomolecules such as proteins and nucleic acids to carboxyl groups on the surface of Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles is easily accomplished using standard carbodiimide coupling technology.

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

- protein purification
- positive separation
- cell separation

Visit our application library *cytiva.com/solutions/genomics* for a list of published articles referencing the Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles.

7 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 and Sera-Mag SpeedBead Carboxylate-Modified 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 and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles are stable in a wide pH and temperature range commonly used in molecular biology such as guanidinium lysis buffers and PCR thermal cycling temperatures.

Long-term colloidal stability of coated particles requires development of an appropriate storage buffer. The selection of storage buffer and pH is critical to achieve optimal particle performance. For stabilizing particle preparations while permitting specific agglutination reactions to occur, use:

- zwitterionic buffers (e.g., MOPSO)
- blocking proteins e.g., bovine serum albumin (BSA) or fish skin gelatin (FSG)
- higher pH
- detergents
- sodium salicylate

In addition, to enhance colloidal stability, proteins with high negative charge, such as BSA and FSG, may be used to block the particle surface against nonspecific sample adsorption. FSG gives good result with antibody-coated particles.

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 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.

Sterilization

Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles are manufactured using proprietary manufacturing processes under strict QSR practices, but the beads are not provided sterile. The formulation contains sodium azide (0.05%) as a preservative to prevent microbial growth.

It is recommended to assess bacterial contamination after long term storage of the particles. Bacterial contamination can be assessed by plating on appropriate growth medium and by checking the plates after 72 hours.

The beads can be sterilized by washing with 70% ethanol. Do not autoclave the beads as this can cause partial to severe beads aggregation.

Protein-conjugated beads can not be sterilized.

Chemical compatibility

Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles are compatible with urea (6 to 8 M) and EDTA.

Drying

Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles can be dried down and some level of aggregates should be expected. Optimize the drying of the beads for your specific application.

Contact *cytiva.com/contact* to dry down beads in lyophilization excipients as custom service.

Preventing aggregation

To prevent aggregation of the particles, avoid the following conditions:

- buffers with extreme acidic pH (carboxyl and sulfonate groups protonation)
- buffers with extreme basic pH (amine groups deprotonation)
- freezing temperatures
- heating
- excessive vortexing
- sonication (especially for protein-labelled magnetic beads)
- microbial contaminants

8 Protein conjugation

8.1 Overview

Introduction

Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles have carboxylic acid groups for covalent coupling via carbodiimide activation (e.g., EDAC). Primary amino groups of nucleic acids, peptides, proteins or other target molecules can be coupled to the bead surface for isolation and affinity purification of biomolecules.

Follow the protocols in the next sections to conjugate a target protein to the particles using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC).

One-step conjugation protocol description

The one-step conjugation protocol is the simpler protocol and is the default method for conjugating species which do not contain carboxyl groups in addition to the target amino groups. This includes amino-functionalized oligonucleotides and some small peptides, plus a wide range of amino-functionalized chemical reagents and linker molecules. If the coating species is applied in a limited amount, conjugation can be reasonably efficient to give a partial monolayer on the particle surface. Applying the coating species in an excess quantity will give less efficient usage, but will result in a more complete layer coating over the particle surface.

EDAC coating chemistry is more efficient when run at lower pH levels and at elevated temperatures. Some species may not be tolerant of these conditions and less optimal conditions may need to be used to preserve their integrity.

Two-step conjugation protocol description

The two-step conjugation protocol is a more complex process, but it does separate out carboxyl group activation from species coating onto the pre-activated carboxyl groups. As such, carboxyl group activation can be run at more optimal low-pH conditions, whilst species coating can be run at higher (milder) pH conditions for pH-sensitive species.

Choice of the conjugation protocol

For coating with proteins, the two-step conjugation protocol is useful if the coating species contain carboxyl groups in addition to the target amino groups. Switching to the two-step process can limit the coating to a monolayer: this may result in less efficient coating (less of the applied protein ends up attached to the particles), but the attached proteins may be presented in a sterically more favourable disposition for later target binding or chemical functionality.

Coating using the one-step process gives a multilayer coating effect, as protein-protein links can form in addition to the desired protein-particle links.

General tips for conjugation protocols

- Pre-wash the particles before any coating process by adding 5× reaction buffer to the particles.
- Optimize the amount of EDAC and protein.
- When adding the protein to the particles, rapid mixing is critical for even coating:
  - at a 1 mL scale: pipette the protein stock directly into the buffered particles. Use the same pipette tip to “syringe” the solution (mix up and down quickly).
  - at a larger scale: place the particles in a beaker with an overhead mixer. While mixing well, add the protein stock quickly into the middle of the vortex.
- Sonicate the particles during/after mixing to achieve a uniform suspension.
- For optimization scale (1 mL), run coupling reactions in 2 mL microcentrifuge tubes. With microtube magnetic racks, coated particles are pelleted to the magnet in 10 to 30 s.
- The particles might clump during coupling due to the electrostatic effect of the positively charged EDAC molecules, the effect of the protein itself, or consumption of negative charge by amide bond formation. Wash into fresh buffer to remove EDAC and unbound protein, then sonicate to reverse the clumping.
- Follow the general washing procedure described in the next section for each wash in the conjugation protocol.

General washing particles procedure

Follow the steps below to wash the particles.

Step	Action
1	Add the buffer.
2	Vortex for 15 seconds or a time sufficient to resuspend the pellet.
3	Separate the particle using a magnetic stand.
4	Aspire the cleared supernatant to waste.

8.2 One-step protein conjugation

About this procedure

The following protocol describes the one-step covalent coupling procedure for 20 mg of Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (0.4 mL of formulation suspension) to a target protein using EDAC.

General considerations for the one-step protein conjugation

The following parameters can be optimized to improve the coupling efficiency.

- **Reaction temperature:** EDAC coatings are generally improved by heating to e.g., 37°C. More heat-resistant proteins can be coated at higher temperatures e.g., up to 50°C for streptavidin.
- **Reaction buffer pH:** EDAC performs optimally at low pH values. With proteins that can tolerate lower pH values, such as streptavidin, the pH of the buffer A stock can be lowered from pH 6.0 as low as 4.5.
- **Reaction volume:** coatings can be improved in some cases by decreasing the overall reaction volume, whilst holding all input reagents the same. Reduce the volume of water used for washed particle resuspension, and possibly also reduce the volume of the protein aliquot keeping mass of applied protein constant.

Additional materials required

- Coupling reagent - Buffer A: 0.5 M MES, pH 6.0
- 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC)
- Storage Solution (any buffer of choice)
- Stock solution of the target protein

Note: *PBS can affect the coupling outcome. If the stock solution of the target protein contain PBS, consider desalting or buffer exchange before use.*

Pre-reaction wash

Follow the steps below to prepare Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles for the coupling reaction.

Step	Action
1	Place the desired amount of particles (about 0.4 mL) into a suitable container e.g., 2 mL microcentrifuge tube (low protein binding tubes are preferred).
2	Add 100 µL of Buffer A.
3	Mix (roll or mechanically mix e.g., via hula mixer) for 15 min at room temperature.
4	Magnetically separate and aspirate the cleared solution to waste.
5	Wash the particles two times with 1 mL water.

The particles are ready for the activation step.

Particle activation and coating

Follow the steps below to activate and coat Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles with EDAC.

Step	Action
1	Resuspend particles with 470 µL of water.
2	Add 200 µL of Buffer A.
3	Dilute protein stock solution in water such that the desired application mass of protein is contained within 250 µL. ***Note:*** *A typical application requires a ratio of protein to particles between 20 to 200 µg protein/mg particle.* *For example, for an applied ratio of protein to particles of 50 µg protein/mg particle, coating 20 mg of particles requires 1 mg of protein. For this to be contained in 250 µl of Buffer A, protein concentration should be 4.0 mg/mL.*
4	Add the prepared protein solution (250 µL) to the particle suspension.
5	Vortex mix to homogenize the mixture.
6	Dilute EDAC in water at 10% w/w (use 1 mL water for every 0.1 g of EDAC). Immediately add 80 µL of the EDAC solution to the reaction tube.

Step	Action
7	Mix (roll or mechanically mix e.g., via thermomixer) for 3 hours at room temperature. ***Note:*** *This step can be performed at higher temperature if your protein is heat tolerant e.g., streptavidin.*
8	At the end of reaction time, magnetically separate and aspirate the reaction solution to waste.

The particles are coupled with the target protein.

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). ***Tip:*** *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.* ***Tip:*** *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.*
2	Resuspend to final desired particle concentration with storage solution. ***Note:*** *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.*

8.3 Two-step protein conjugation

About this procedure

The following protocol describes the two-step covalent coupling procedure for 20 mg of Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (about 0.4 mL of formulation suspension) to a target protein using EDAC.

General considerations for two-step protein conjugation

The reaction volume can be optimized to improve the coupling efficiency. The activated carboxyl groups are very susceptible to de-activation via hydrolysis under the more alkaline coating conditions. Keep the protein concentration higher than 5 mg/mL to compete with water for the activated carboxyls (critical for high levels of coating).

Additional materials required

- Coupling reagents:
  - Buffer B: 0.5 M MES (2-(*N*-morpholino)ethanesulfonic acid), pH 4.5
  - Buffer C: 10 mM MES (dilute 1 part buffer B to 49 parts water)
  - Buffer D (20× stock):1.0 M sodium borate buffer, pH 8.5
- N-Hydroxysuccinimide (NHS)
- 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC)
- Storage solution (any buffer of choice)
- Stock solution of the target protein

Note: *PBS can affect the coupling outcome. If the stock solution of the target protein contains PBS, consider desalting or buffer exchange to Buffer D (1×) before use.*

Pre-reaction wash

Follow the steps below to prepare Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles for the coupling reaction.

Step	Action
1	Place the desired amount of particles (about 0.4 mL) into a suitable container e.g., 2 mL microcentrifuge tube (low protein binding tubes are preferred).
2	Add 100 µL of Buffer B.

Step	Action
3	Mix (roll or mechanically mix e.g., via hula mixer) for 15 min at room temperature.
4	Magnetically separate and aspirate the cleared solution to waste.
5	Wash the particles twice with 1 mL water.

The particles are ready for the activation step.

Particle activation

Follow the steps below to activate Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles with EDAC.

Note: *As the activation step does not include protein, the pH of the reaction is kept low (pH 4.5) for optimum EDAC activation of particle carboxyl groups.*

Step	Action
1	Resuspend particles with 590 µL of water.
2	Add 200 µL of Buffer B.
3	Dilute NHS in water at 1 mL water for every 0.1 g NHS. Add 50 µL of this solution to the reaction tube.
4	Vortex mix to homogenize the mixture.
5	Dilute EDAC in water at 10% w/w (1 mL water for every 0.1 g of EDAC). Immediately add 160 µL of the EDAC solution to the reaction tube.
6	Mix (roll or mechanically mix eg via thermomixer) for 30 min at room temperature.
7	At the end of activation time, magnetically separate and aspirate the reaction solution to waste.

The particles are activated.

Interstep washing

In most cases, aspiration of the activation mix removes enough of the excess reagents to be acceptable. For two-step coating, the activated beads can be washed before proceeding to coating. Optimize the interstep washing if required for a specific application.

Note: *EDAC-activated carboxyl groups are not particularly stable; washing steps should be conducted sparingly and quickly.*

Wash activated particles 1 to 3 times with Buffer C (1.0 mL each wash) to remove excess reagents.

Note: *The pH of the wash solutions should remain low to preserve the activated carboxyl groups as far as possible. If just one wash is used, this can be water instead of Buffer C; the residue of Buffer B in the wet bead pellet will maintain a low pH.*

Note: *If more washes are used, water is not desirable as the pH will rise as activation buffer is washed out.*

Note: *Do not use phosphate buffer for interstep washes.*

After washing, the particles are ready to be coated. Immediately proceed with the coating steps.

Particle coating

Follow the steps below to coat Sera-Mag and Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles with the target protein.

Step	Action
1	Dilute the target protein stock solution in water and add Buffer D (20×) such that the desired application mass of protein is contained within 250 µL of Buffer D (1×). ***Note:*** *A typical application requires a ratio of protein to particles between 20 to 200 µg protein/mg particle.* *For example, for an applied ratio of protein to particles of 75 µg protein/mg particle, coating 20 mg of particles requires 1.5 mg of protein. For this to be contained in 250 µl of Buffer D (1×), protein concentration should be 6.0 mg/mL.*
2	Add the prepared protein solution in Buffer D (1×) (250 µL) to the particle suspension. Make sure to mix rapidly to disperse the particles.
3	Mix (roll or mechanically mix e.g., via thermomixer) for 60 minutes at room temperature.
4	At the end of reaction time, magnetically separate and aspirate the reaction solution to waste.

The particles are coupled with the target protein.

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). ***Tip:*** *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.* ***Tip:*** *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.*
2	Resuspend to final desired particle concentration with storage solution. ***Note:*** *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.*

8.4 Determination of coupling efficiency

Different methods can be used to check binding of ligands to our beads:

- **Optical Density (OD) measurement (indirect quantification)**: measure the OD of the ligand before immobilization to the beads and compare it with the ligand concentration that is left in the supernatant after coating. This gives a crude measurement of how much protein that has bound to the beads.
- **Fluorescent labelling (indirect quantification)**: add labelled ligand to the beads, measuring how much ligand is left in the supernatant (i.e., not bound to the beads) compared to the original sample.
- **Radioactive labelling**: label radioactively a portion of the ligand in tracer amounts and mix it with "cold" ligands in a known ratio before coupling. Measure the beads in a scintillation (gamma) counter for absolute quantities of ligand by comparing the counts per minutes with a standard.
- **Bicinchoninic acid (BCA) assay**: perform the assay following manufacturer instructions. For the assay standard curve, the BSA reference provided in the kit can be used, but for best accuracy create the assay standard curve using the target protein used for coating. Uncoated carboxylate-modified beads give a small response to this assay. Make sure to take into account the uncoated particle response to quantify the coupling efficiency.

9 Troubleshooting

Problem	Possible cause	Corrective action
Magnetic particles aggregated	Long storage or magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions for use.
Clumping during coupling reaction	Buffer was incompatible with magnetic beads	Isolate the step that causes clumping.
	Carbodiimide addition	• Add EDAC quickly while mixing e.g., pipette mixing as it is added or immediate rapid vortexing to quickly homogenize the mixture. • Decrease bead concentration.
	Protein addition	Increase protein concentration.
	Washing	Add surfactant or reduce number of washing steps.
Low binding	Suboptimal reaction condition	Move pH closer to protein isoelectric point.
Variable coating	Suboptimal coupling condition	• Use pure water – no contaminants. • Use fresh reagents.
Protein leaches with long-term storage	Suboptimal coupling condition	Try covalent attachment or lyophilize final product.
Beads 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 the suspension prior to centrifugation. Use low protein binding microcentrifuge tubes.

10 Related products

Product name	Product code
MagRack 6	28948964
MagRack Maxi	28986441
Magnetic Separation Rack 15 mL	29710714

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