Sera-Mag™ SpeedBeads Carboxylate-Modified Magnetic Particles

SAMPLE PREPARATION AND NUCLEIC ACID ISOLATION

Sera-Mag™ SpeedBeads Carboxylate-Modified Magnetic Particles are nominal 1 µm magnetic particles of uniform size that feature a second layer of magnetite (Fig 1). As a result, Sera-Mag™ SpeedBeads respond much faster to a magnetic field to separate quickly and completely from suspensions. This ensures shorter assay times in clinical diagnostic tests as well as faster particle movement through viscous solutions.

- Fast reaction kinetics increases throughput and precision.
- Low nonspecific binding improves assay accuracy.
- Cauliflower-like surface increases overall surface area and binding capacity.
- Carboxylic groups on the surface permit easy covalent coupling using simple carbodiimide chemistry (Fig 2).
- Salt-tolerance and slow settling rate provide excellent colloidal stability in the absence of a magnetic field.
- Uniform diameter provides excellent lot-to-lot reproducibility.
- Surfactant-free particles require no washing.
- Stability in buffer systems and detergents allows versatility in reagent and sample preparation.

Sera-Mag™ SpeedBeads Carboxylate-Modified Magnetic Particles are useful as a solid phase support for many applications including sample preparation, nucleic acid isolation and immunoassay applications due to their speed, precision and increased binding capacity. Prior to downstream applications, samples can be mixed with the magnetic particles, whereupon biomolecules of interest are covalently attached to carboxyl groups on the particles' surfaces. Isolation or purification of the biomolecules occurs through magnetic separation. The carboxyl groups on these particles are activated by the water soluble carbodiimide 1-ethyl-3-(3 dimethylaminopropyl)-carbodiimide (EDAC). Once activated, the groups react with the free amino groups of the adsorbed protein to form amide bonds. If exposure to EDAC is found to be potentially harmful to the protein, then we recommend using our two-step covalent coupling procedure which prevents this.

Fig 1. SEM image showing the cauliflower-like surface of the Sera-Mag™ SpeedBeads that dramatically increases the overall surface area available for binding.

Sera-Mag™ beads are available in the original Sera-Mag™ version (with single magnetite layer) and the SpeedBeads version (with dual magnetite layer). Both bead types provide high sensitivity and low non-specific binding for greater accuracy.

These carboxylate-modified magnetic particles also have a distinct cauliflower-like surface that adds to the overall surface area available for binding and without any reduction in particle size.

Modified core shell process

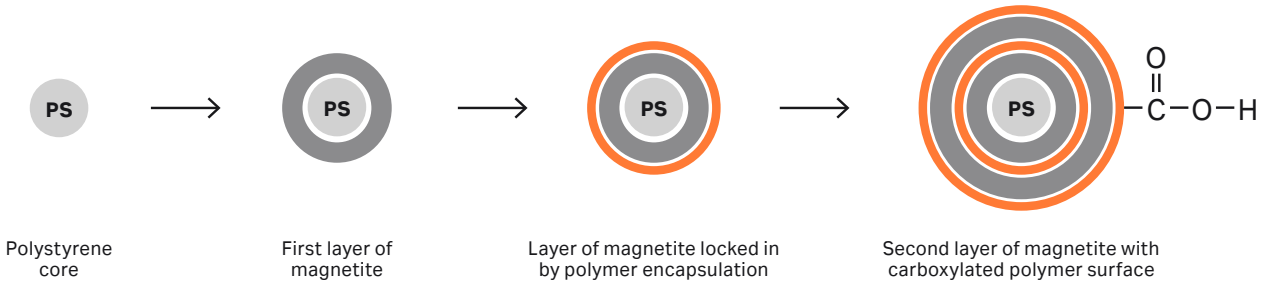

Fig 2. Sera-Mag™ SpeedBead Carboxylate-Modified Magnetic Particles feature carboxylic groups on the surface that permit easy covalent coupling using simple carbodiimide chemistry.

Settling parameters

When integrating magnetic particles into any workflow or assay platform, it is important to understand how those particles act in solution. Settling rates and speed to magnet are two important factors, as incomplete capture or extended time to magnet can affect assay results. Sera-Mag™ SpeedBeads Carboxylate Modified Magnetic Particles are designed for fast attraction to the magnet due to their additional coating of magnetite when compared to original Sera-Mag™ particles (Fig 3). This can be advantageous when time is important or when using more viscous sample. Note that magnetic particles in a magnetic field interact with each other, with time to magnet decreasing as bead concentration increases.

When handling magnetic beads, either in production or end use application, the speed of gravity settling must also be considered. Sera-Mag™ SpeedBeads Carboxylate show slow rates of sedimentation in aqueous suspensions over time (Fig 4). This allows them to be sampled repeatedly from a stock volume over time with reduced need for mixing, but also enables consistent, precision performance in automated platforms.

For dilute suspensions, bead concentration in suspension can be conveniently monitored by light scattering. Use absorbance as a measure of particle concentration via sample turbidity, if using detection at longer wavelengths e.g. 700 nm.

Reagent compatibility

Sera-Mag™ SpeedBeads can be used across a wide array of applications including nucleic acid isolation and proteomics where there is often a need for chemical stability e.g. during conjugation. Sera-Mag™ SpeedBeads Carboxylate are stable across a range of pH, buffers and detergents. Table 1 provides examples of typical use conditions.

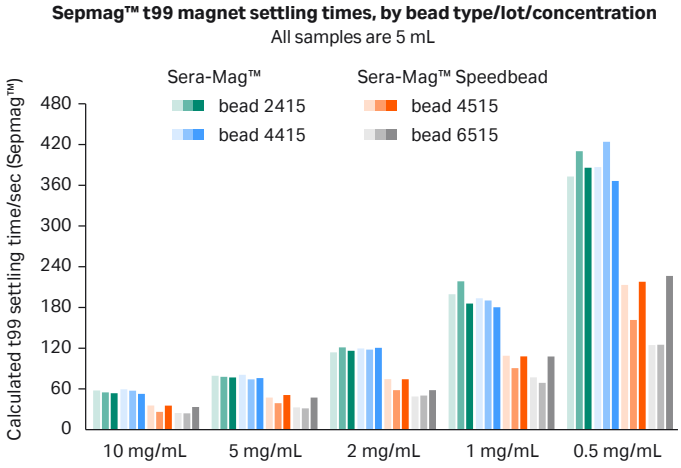

Fig 3. Sepmag™ A 400mL (Sepmag) was used to measure settling rate of Sera-Mag™ SpeedBeads to the magnet. The graph shows the settling of SpeedBeads to concentrations between 0.5–10 mg/mL. As the concentration decreases the settling time slows.

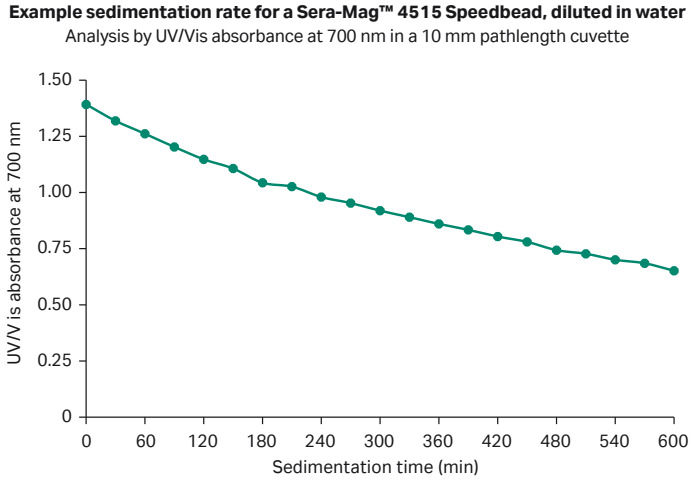

Fig 4. Sedimentation rate of Sera-Mag™ SpeedBeads Carboxylate suspended in water measured at 700 nm. A volume of 3.5 mL sample in a cuvette (liquid column volume ~ 35 mm high), with detection beam at a height of 8.5 mm above the bottom of the cuvette.

Table 1. Typical use conditions for Sera-Mag™ SpeedBeads Carboxylate

Reagent / condition	Compatibility			Comments
	Low	Medium	High	
Low pH, ≥ 3			x	Activation and/or conjugation of carboxyl groups in aqueous buffers using 1-Ethyl-3-(3-Dimethylaminopropyl) carbodiimide (EDAC), effective with exposure for several hours. Example buffer components include 2-(N-morpholino) ethanesulfonic acid (MES), pyridine. Also carboxylic acid buffer e.g. saline-sodium citrate (SSC) in nucleic acid isolation processes can be included.
Extreme low pH, ≤ 3	x			Prolonged exposure not recommended; risk of dissolution of iron oxide component.
High pH, ≤ 11			x	Reaction of base carboxylate beads with bis-amine linkers; onward reaction of bead-linked amine groups with anhydride and epoxide reagents. Effective with exposure for several hours. Example buffer components include carbonate, borate, phosphate and phosphate buffered saline (PBS), plus dilute alkali hydroxide.
Extreme high pH, ≥ 11		x		Prolonged exposure not recommended; risk of degradation of polymer encapsulation.
Ethanol, 70% –100%		x		Suitable for short-term exposure, for example as used as wash solution in nucleic acid isolation processes.
Isopropanol, 70%–100%		x		Suitable for short-term exposure, for example as used as wash solution in nucleic acid isolation processes.
Acetonitrile		x		Suitable for short-term exposure, for example as used as binding and wash solutions in single pot solid phase sample prep (SP3) protein isolation processes. Prolonged exposure not recommended; risk of swelling and/or degradation of polymer core.
Guanidinium salt solutions (GuHCl and GuSCN)		x		Suitable for short-term exposure, for example as used as lysis and binding solutions in nucleic acid isolation processes. Prolonged exposure not recommended; risk of dissolution of iron oxide component.
Chelators e.g. EDTA			x	Suitable for longer term exposure in formulation, for concentrations ≤ 1 mM. Suitable for short-term exposure, e.g. in nucleic acid isolation processes, for concentrations ≤ 10 mM.
Detergents e.g. TWEEN™-20, Triton™ X-100			x	Suitable for longer term exposure in formulation, as recommended for protein-coated beads.
Sodium azide			x	Suitable for longer term exposure in formulation, for concentrations ≤ 0.1% w/w, as used for microbial control.
PEG (polyethylene glycol)		x		Suitable for medium-term exposure, for example as used in binding solutions in solid phase reverse immobilization (SPRI) nucleic acid isolation processes.
DMF	x			Not recommended
DMSO	x			Not recommended

Conjugation

Sera-Mag™ SpeedBeads Carboxylate are often modified for specific assays that require a defined ligand to be attached to the surface of the bead. These conjugations are typically undertaken using EDAC activating reagent in aqueous buffer. The simplest format is to use a one-step process where most of the applied protein sample can be attached to the beads in a multi-layered arrangement. This allows high levels of protein to be attached to the surface, but the protein may not necessarily be available for subsequent binding of larger target molecules due to steric interference.

An alternative approach is to use a two-step coupling procedure that results in a monolayer of protein. This can also be achieved using EDAC activating reagent if the coating process is amended to a two-step process, where the Sera-Mag™ carboxyl groups are first activated with EDAC and subsequently allowed to conjugate with protein amino groups. This approach can eliminate protein-protein crosslinking and provide a complete protein coating, more of which is available to bind target molecules. EDAC coating using the two-step process is more representative of the binding capacity of the bead itself.

Refer to *Covalent coupling procedures for Sera Mag™ and Sera-Mag™ SpeedBeads Carboxylate-Modified Magnetic Particles* regarding conjugation methods with EDAC.

As an indication of how much protein can be bound to the surface of Sera-Mag™ Carboxylate beads, we can look at conjugation of streptavidin to the surface by the two-step process. When all the various protein assay techniques are considered, there is a level of agreement in the limit of protein binding to a Sera-Mag™ bead of around 85–100 µg per mg bead.

Specifications

	Sera-Mag™ SpeedBeads	Sera-Mag™
Particle composition	Double layer of magnetite	Single layer of magnetite
Particle density	~2.0 g/cm^3	~1.7 g/cm^3
Content		
Magnetite	~60%	~40%
Acid	High acid content with parking areas ranging from ~2 to 5 Å^2 per carboxyl group	
Product attributes		
Nominal diameter	1 µm	1 µm
Concentration	Supplied at approximately 5% solids (50 mg/mL)	
Additives	0.05% sodium azide	0.05% sodium azide
Storage and handling	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, roller or vortex mixer.	

Ordering information

Product	Quantity	Product code
Sera-Mag™ SpeedBead Carboxylate-Modified [E7] Magnetic Particles	15 mL	45152105050250
	100 mL	45152105050350
	1000 mL	45152105050450
Sera-Mag™ SpeedBead Carboxylate-Modified [E3] Magnetic Particles	15 mL	65152105050250
	100 mL	65152105050350
	1000 mL	65152105050450
Sera-Mag™ Carboxylate-Modified [E7] Magnetic Particles	15 mL	24152105050250
	100 mL	24152105050350
	1000 mL	24152105050450
Sera-Mag™ Carboxylate-Modified [E3] Magnetic Particles	15 mL	44152105050250
	100 mL	44152105050350
	1000 mL	44152105050450

What is the difference between E3 and E7 bead?

E3 and E7 refer to the different manufacturing process for the beads. E3 and E7 beads behave similarly and we continue to provide both bead types to our customers for test and validation in their chosen application.

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

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