Sera-Mag™ speedbead carboxylate-modified 3 µm magnetic particles

Sera-Mag™ speedbead carboxylate-modified 3 µm magnetic particles are magnetic beads that are roughly spherical particles with mean diameters of approximately 2.7 to 3.2 µm. The particles have available carboxylic acid chemical functional groups on the bead surface.

Key features:

- Fast reaction kinetics increase throughput and precision.
- Cauliflower-like surface increases overall surface area and binding capacity, above that expected for a smoothly spherical particle of the same diameter.
- Carboxylic acid groups on the surface permit easy covalent coupling using simple carbodiimide chemistry to species bearing primary amino groups.
- Salt-tolerance and slow settling rate provide good colloidal stability in the absence of a magnetic field.
- Uniform particle diameter between 2.7 to 3.2 µm provide excellent lot-to-lot reproducibility.
- Surfactant-free particles require no washing.
- Stability in buffer systems and detergents allows versatility in reagent and sample preparation.

3 µm carboxyl bead structure and size

Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles are formed around uniform, polystyrene-based core particles. These core particles are combined with magnetite (magnetic iron oxide) nanoparticles and an encapsulation polymer mix to coat the polystyrene core particles with a magnetically responsive composite surface layer. Like Sera-Mag 1 µm speedbeads, two layers of this composite coating are incorporated onto the core particles to enable fast response to an applied external magnetic field (Fig 1).

Modified core-shell process

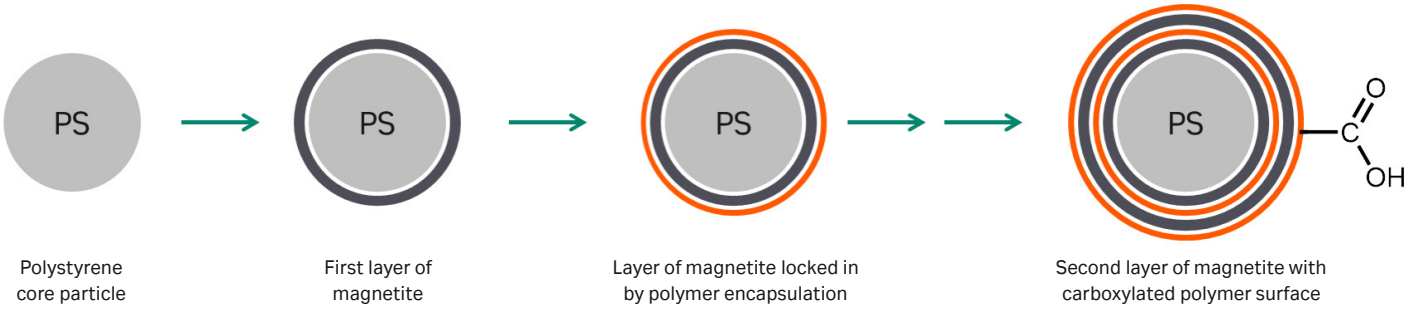


Fig 1. The core-shell construction process for Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles. The finished particles exhibit surface carboxylic acid groups that permit easy covalent coupling via carbodiimide chemistry.

Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles can be useful as a solid phase support for many applications including sample preparation, nucleic acid isolation, and immunoassay applications:

- Prior to downstream applications, samples can be mixed with the magnetic particles. The biomolecules of interest can be permanently attached to carboxyl groups on the particles' surfaces via covalent chemical bonding (mediated by reactive agents e.g., carbodiimides). Particles so modified then have specific required properties for the chosen application.
- In other applications, samples can be simply mixed with the magnetic particles. The biomolecules of interest can be reversibly bound to the particles' surfaces via electrostatic bonding. Such reversible bonding is applicable to isolation and purification processes, typically operating via a bind-wash-elute mechanism.

Isolation or purification of the biomolecules occurs through operations involving magnetic separation. Such magnetically controlled operations are typically simpler and faster than more traditional nonmagnetic operations such as filtration, chromatography, centrifugation, etc. Magnetic particles are not required to be contained within specific device constructs such as filters or columns but can be handled directly in suspension within process tubes or plates. Magnetically controlled operations are thus well suited to high-throughput process formats.

The magnetic beads are approximately spherical and have a mean diameter that has a peak in the range of 2.7 to 3.2 µm on a size-frequency histogram. The size of the beads was determined via transmission electron microscopy (TEM). Frequency histograms of the bead sizes exhibit an approximately normal distribution within the size range specifications (Fig 2). Bead morphological studies show the cauliflower-like rough surface typical of Sera-Mag beads. This surface confers a larger surface area for binding interactions than the surface area of a smoother, more regularly spherical bead of the same diameter.

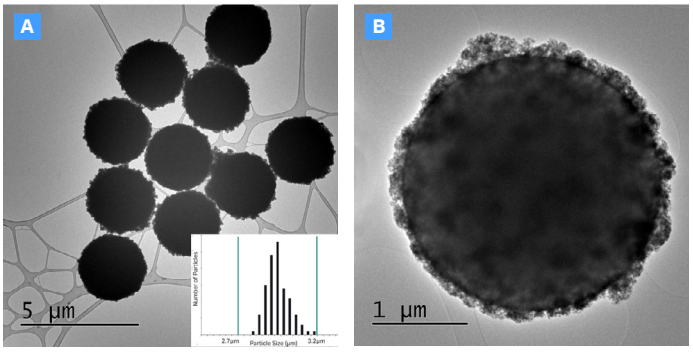


Fig 2. Example imaging and sizing results for the Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles. (A) example bead lot with a mean bead diameter of 2.90 µm and a standard deviation of 0.07 µm and coefficient of variation of 2.96%. The green lines on the graph represent the target range for the bead size (peak mean diameter). (B) A higher magnification TEM image of a single bead highlighting the classic Sera-Mag cauliflower-like surface morphology. All samples were analyzed via TEM at ~ 5 mg/mL in pure water.

Magnetic response

When integrating magnetic particles into any workflow or assay platform, it is important to understand the performance of the particles in solution. Speed to magnet is one important factor for this because incomplete capture or extended time to magnet can affect assay results. Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles are designed for fast attraction to the magnet due to their additional coating of magnetite, which is comparable in magnetic performance to original Sera-Mag speedbead 1 µm particles. This performance can be advantageous with time-dependent experiments and more viscous samples.

Obtaining numerical data for magnetic response for characterization and comparative purposes is not normally practical with most common types of magnetic separators, but certain commercially available separators are designed for this purpose. An example is the Sepmag A400ml magnet separator operated with its associated software. This unit provides a controlled magnetic field environment for reproducible separations. The Sepmag A400ml unit uses in-built LED lights and an associated detector to measure the optical density (~ absorbance, reported as suspension homogeneity) of the particle suspension. This value is recorded over the course of a magnetic separation. The operating software will automatically fit model sigmoid curves to the raw response curves and report out associated numerical values to enable comparative analyses between runs.

Figure 3 shows example raw data and normalized views for a typical lot of Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles suspended in water at varying concentrations.

Sepmag A400 raw data, normalized response curves

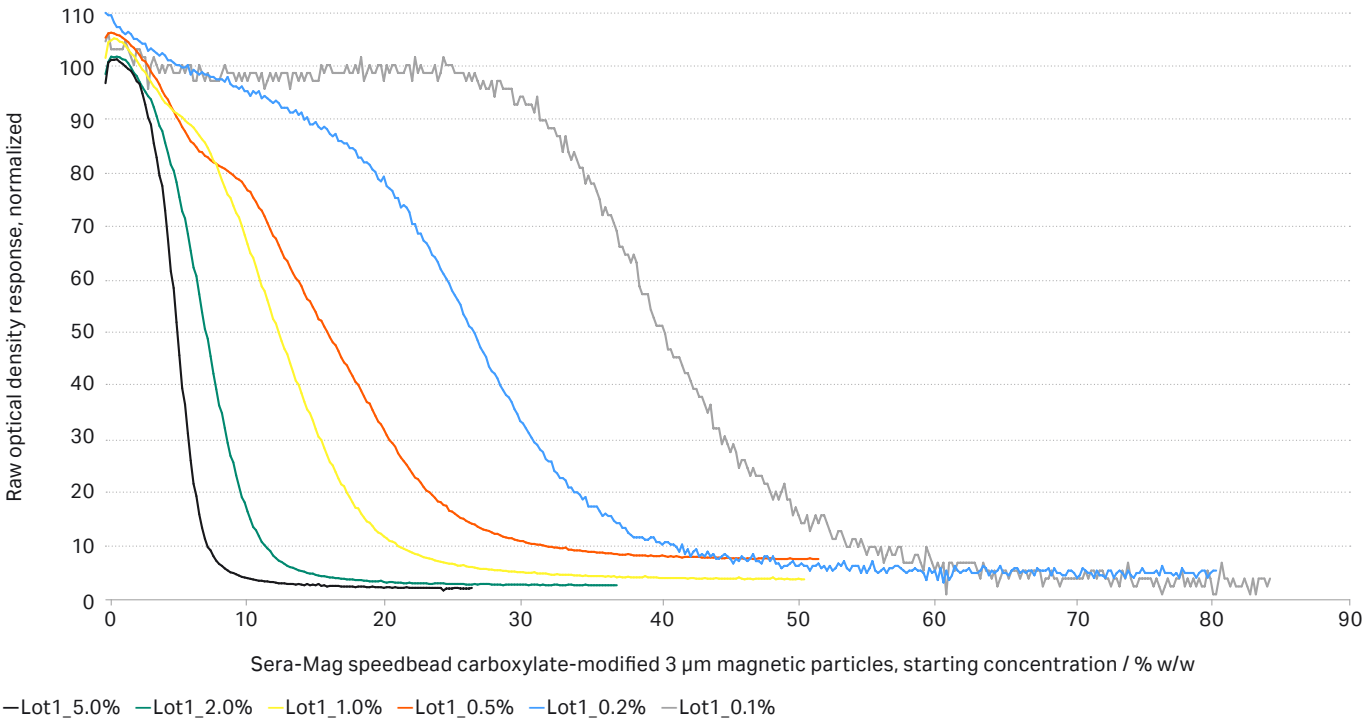


Fig 3. A normalized graph view of example magnetic response curves with decreasing bead concentration using the Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles. Magnetic response was measured using a Sepmag A400ml magnetic separator. Beads were analyzed in pure water using 1.8 mL sample volumes in 75 × 10 × 0.8–1.0 mm glass tubes in the Sepmag A400ml analytical insert 2 mL tube position. The plots are individually scaled so that starting V_{max} values are scaled to 100% and terminal V_0 values are scaled to 0%. Normalized data values are yielded by data export from the operating software to Excel. Note that the general slowing of particle magnetic clearance as the particle starting concentration decreases is typical.

In an applied external magnetic field, the particles are not only attracted towards the magnet but are also magnetized and attract each other. The resulting magnetic aggregates move more rapidly to the magnet than isolated particles. This magnetic aggregation is promoted by higher particle concentrations, which is why more concentrated suspensions show a more rapid depletion. The magnetic aggregation effect can be observed when viewing bead suspensions on a slide under a light microscope if a small magnet is placed nearby (Fig 4).

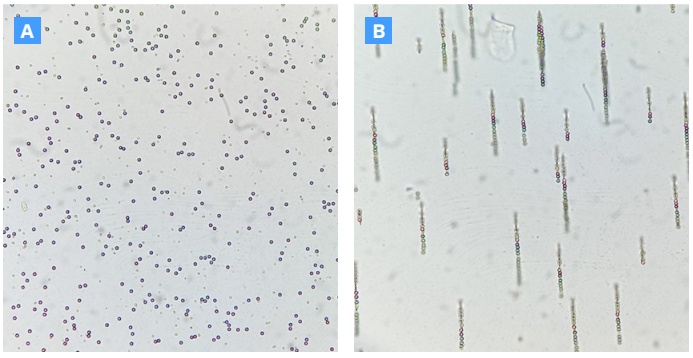
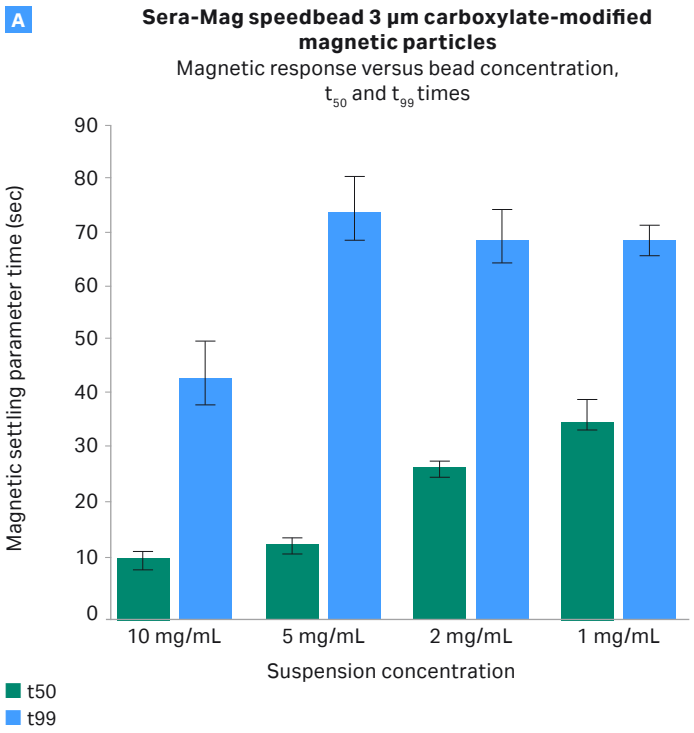


Fig 4. Behavior of Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles: magnetically-induced aggregation as observed under a light microscope. (A) The particles as observed on a glass slide with no external magnetic field. The particles are evenly dispersed. Small groups of two or more particles may form over time, but these are not permanent aggregates and are easily separated by tapping on the coverslip. (B) The same sample after a small magnet is placed next to the slide. The particles are attracted to each other and form larger aggregates because of their induced magnetism. These aggregates form chains that align with the external field. Upon removal of the magnet, the particles lose all induced magnetization (superparamagnetism) and are again easily dispersed by any subsequent mechanical agitation.

To determine the actual time required for effective particle clearance, a value t_{99} can be derived the fitted curve's reported t_{50} and P values. The t_{99} value represents the finite time taken to clear 99% of particles from suspension. (Note that, for a fitted sigmoid curve, the time taken for total 100% clearance will, by mathematical definition, always be infinite).

Figure 5 shows fitted t_{50} and calculated t_{99} clearance times for a typical lot of Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles suspended in water at varying concentrations. Note that, in the uniform-field arrangement of the Sep-Mag particles, the t_{50} values show a steady increase as bead concentration falls. This increase is related to the reduced number and size of magnetic aggregates that form during separation because the particles start farther apart in more dilute suspensions. But the t_{99} values show the effective clearance time reaches a plateau as bead concentration falls. This plateau is related to the tail of the separation that involves straggling, lone particles. As the particles are fairly uniform in size, the uniform magnetic field pulls on these isolated stragglers with a similar force causing them to move at a similar speed. As a result, the effective clearance is completed in similar times. Note: this effect is observed due to the particularly uniform field arrangement in this separator. For more commonly encountered separators, which use single localized magnets, this effect may not be apparent.

A comparison with an equivalent 1 µm Sera-Mag carboxyl bead under a single representative condition is also shown.



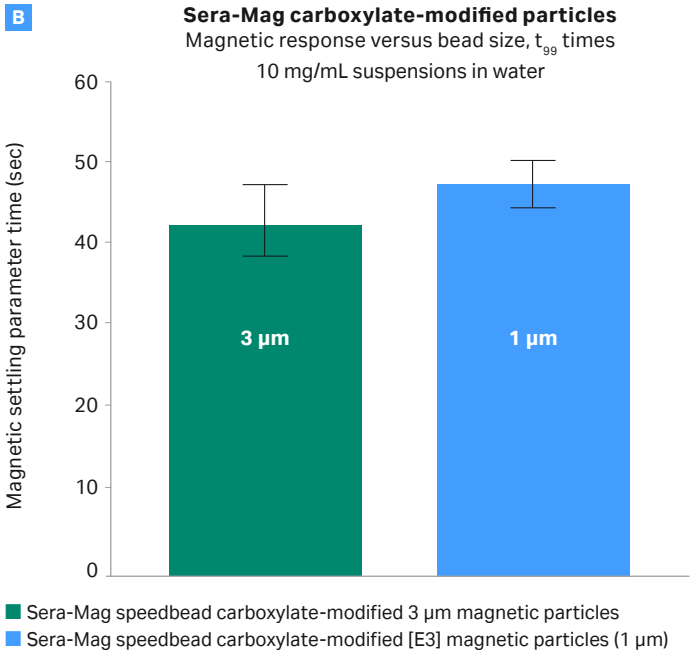


Fig 5. Effective clearance times for Sera-Mag carboxylate-modified 3 µm magnetic particles. (A) Calculated t_{50} and t_{99} clearance values for four starting 3 µm particle concentrations. (B) Comparison of t_{99} clearance value for the 3 µm particle versus an equivalent Sera-Mag 1 µm carboxyl speedbead particle at one representative starting concentration. Clearance times are comparable between the two bead sizes.

Beads were analyzed in pure water using Sepmag A400ml magnetic separator with triplicate samples, and each sample was measured on the machine in triplicate. Columns thus represent n = 9 sample average values, with standard deviation error bars included. The t_{50} and P values are generated automatically from a model sigmoid curve that is fitted to the raw data within the Sepmag software. A t_{99} value is calculated using the formula $t_{99} = t_{50} \times 99^{(1/P)}$ (from the Sepmag user manual) and represents the time required for 99% clearance of beads from suspension.

Gravity settling rate

When handling magnetic beads, the speed of gravity settling must also be considered.

As a cardinal rule for reproducible dispensing a solid particle suspension, the suspension must be fully homogenized beforehand. This can be achieved by vortex mixing small volumes or, more conveniently, by roller mixing larger, bulk volumes. If a homogenized volume is allowed to stand without further agitation, the suspension will slowly become heterogeneous. This behavior is normal because the particles react to gravity with respect to their composite density and that of the fluid medium in which they are suspended. For almost all aqueous-based suspension media, magnetic beads will be significantly denser than the medium due to their dense metal oxide magnetic component. Thus, when left undisturbed, the particles will inevitably settle downward over time due to gravity.

As gravity settling does involve a change from a relatively homogeneous state to a heterogeneous one, obtaining numerical data forms for quantitative comparison between different conditions and beads is difficult and normally impractical. While quantitative methods can be devised to give a graphical plot of settling progress over time, the results may vary greatly depending on exactly how the experiment is set up, making comparisons very subjective. Comparison by qualitative visualization gives a more useful assessment of the time taken for a given suspension to display clear development of heterogeneity. That is, a user can see the bulk suspension clear and a settled particle bed form at the bottom of the container.

While it is best practice to remix magnetic particle formulations between samplings to guarantee reproducibility, visual assessment can give a user an idea of how long a homogenized suspension can stand between samplings before the risk of significantly different aliquots becomes too high. Particle suspensions that settle due to gravity at an unduly rapid rate pose a greater challenge to maintain reproducibility and can be less well regarded by some users as a result.

Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles show relatively slow rates of sedimentation over time in aqueous suspensions. To examine the gravity settling rate of the magnetic particles, the suspension of the beads was examined visually over time. When comparing the Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles to the equivalent 1 µm particles, both particle solutions in water maintained good suspension for 2 h in 6 mL of suspension (Fig 6).

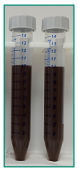
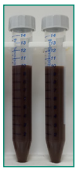
Time (min)	0	120
Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles		
Sera-Mag speedbead carboxylate-modified [E3] magnetic particles (1 µm)		

Fig 6. Gravity settling of Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles versus the equivalent 1 µm magnetic particles shows good suspension is maintained. The photos show 6-mL suspensions in pure water, monitored over 2 h.

Reagent compatibility

Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles 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). The particles are stable across a range of pH, buffers, and detergents. Table 1 provides examples of typical use conditions and reagent compatibilities.

Table1. Typical use conditions for Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles.

Reagent / Conditions	Compatibility			Comments
	Low	Medium	High	
Low pH, 3 < pH ≤ 7			X	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 buffers (e.g., saline-sodium citrate [SSC]) in nucleic acid isolation processes can be used.
Extremely low pH, ≤ 3	X			Prolonged exposure not recommended; risk of dissolution of iron oxide component.
High pH, 7 ≤ 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, phosphate buffered saline (PBS), and dilute alkali hydroxide.
Extremely high pH, ≥ 11	X			Prolonged exposure not recommended; risk of degradation of polymer encapsulation.
Ethanol, 70% to 100%		X		Suitable for short-term exposure, for example, used as wash solution in nucleic acid isolation processes.
Isopropanol, 70% to 100%		X		Suitable for short-term exposure, for example, used as wash solution in nucleic acid isolation processes.
Acetonitrile		X		Suitable for short-term exposure, for example, 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.
Guanidine hydrochloride solution (GuHCl)		X		Suitable for short-term exposure, for example, used as lysis and binding solutions in nucleic acid isolation processes.
Guanidinium thiocyanate solution (GuSCN)	X			Not recommended; risk of dissolution of iron oxide component with thiocyanates (forms red $FeSCN^{2+}$ solution complex).
Chelators (e.g., EDTA)			X	Suitable for longer-term exposure in formulation for concentrations ≤ 1 mM. Suitable for short-term exposure, for example in nucleic acid isolation processes with 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.
Polyethylene glycol (PEG)		X		Suitable for short-term exposure, for example, used in binding solutions in solid phase reverse immobilization (SPRI) nucleic acid isolation processes. Organic ethers have inherent susceptibility to free-radical degradation by aerial oxygen, which can be catalyzed by iron compounds. For longer-term exposure, stability added antioxidant stabilizers may be required or use PEG products that already include stabilizers.
DMF	X			Not recommended
DMSO	X			Not recommended

Conjugation

Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles can also be modified for specific assays that require a defined ligand to be attached to the surface of the bead. Amine containing ligands can be conjugated to the bead surface by using EDAC [1-ethyl-3-(3 dimethylaminopropyl)-carbodiimide] activating reagent in an aqueous solution. The simplest method is to use a one-step process where most of the applied ligand can be attached to the beads in a multilayered arrangement. This arrangement allows high amounts of ligand to be attached to the surface. However, the ligand may not be available for subsequent binding of other target molecules due to steric interference.

As an indication of how protein can be bound to the surface of Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles, streptavidin was conjugated to the surface using the one-step, EDAC conjugation process recommended by Cytiva. A broadly linear response is observed between amount of streptavidin added for conjugation and the biotin-binding to the streptavidin bound to the magnetic beads post conjugation. This result indicates predictable conjugation of streptavidin (Fig 7).

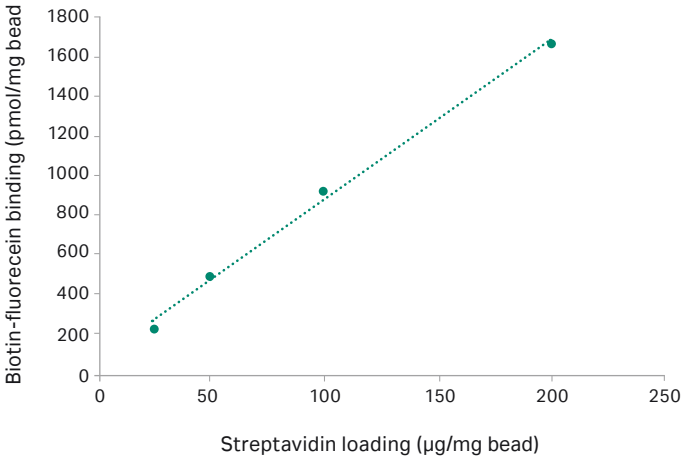


Fig 7. Conjugation of streptavidin onto Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles via Cytiva one-step, EDAC coupling protocol shows a linear relationship between achieved biotin binding capacity vs increasing streptavidin loading.

An alternative approach to conjugation is to use a two-step, EDAC coupling procedure to achieve a monolayer of ligand. The Sera-Mag particle carboxyl groups are first activated with EDAC and subsequently allowed to conjugate with the amino groups of the ligand. This approach can eliminate ligand-ligand crosslinking and provide a complete protein coating with a higher proportion of the coating available to bind target molecules.

Refer to covalent coupling procedures as described in the Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles **Instructions for use** document.

Specifications

Specification	Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles
Particle composition	Double coating of magnetite
Functionalized surface	Carboxylic acid group
Bead mean diameter	2.95 ± 0.25 µm
Magnetite content	~ 25% w/w
Particle density	~ 1.3 g/cm^3
Concentration	5% solids (50 mg/mL)
Suspension medium, plus additives	Purified water, containing 0.05% w/w sodium azide
Storage and handling	Unless otherwise stated, refrigerate product (2°C–8°C) 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

Summary

Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles are 3 µm magnetic beads with the typical Sera-Mag cauliflower-like rough surface for enhanced surface area. Both magnetic response of the particles to an applied magnetic field as well as gravity settling rate are important factors to understand when incorporating magnetic beads into assay workflows. When an applied magnetic field was applied to an aqueous particle solution, these magnetic particles show consistent, fast magnetic response times at different bead concentrations from 1 to 10 mg/mL. The particles also maintain good suspension in an aqueous solution in the absence of an applied magnetic field. Available carboxyl groups on the particle surface also enable amine containing ligands to be conjugated to the surface of the magnetic particles if the downstream application requires ligand attachment to the particles.

Ordering information

Product	Product code
Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles, 1 mL	29729998
Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles, 10 mL	29729997
Sera-Mag speedbead carboxylate-modified 3 µm magnetic particles, 100 mL	29730063

Related products	Product code
Magnetic separation rack 15 mL	29710714
MagRack 6	28948964
MagRack Maxi	28986441

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