Sonication and mixing of Sera-Mag™ magnetic particles

Sera-Mag™ and Sera-Mag speedbeads provide a cost-effective magnetic bead separation technology for molecular biology applications, nucleic acid isolation, and immunoassays. The particles feature a large surface area, offering high sensitivity and physical stability. Our product range includes carboxylate-modified particles, as well as amine-blocked and protein A/G, oligo(dT), streptavidin -, and Neutravidin-coated versions.

Introduction

Effective processing is one of the most critical aspects of magnetic particle utilization. Monodispersity and homogeneous suspension of particles should be carefully controlled during use to ensure robust and reproducible performance. This procedure discusses methods for successful use of Sera-Mag magnetic particle technology.

Sonication overview

Sonication provides a way to resuspend Sera-Mag particles thoroughly and efficiently without damaging them. After centrifugation, processing steps, and coupling reactions, we recommend sonication for most particles to avoid difficulties that arise from improper particle resuspension.

We routinely sonicate our coated particle preparations with a probe-type ultrasonicator to resuspend pellets after centrifugation and to reverse mild aggregation induced by coupling. We have not found this to be detrimental to sensitized particles in any way; we have even seen improvement in sensitivity after sonication. However, sonication may damage ligand-coupled particles. If damage from sonication is a concern, we recommend slow vortexing.

Using Sera-Mag streptavidin-coated particles, we tested whether sonication caused desorption of proteins or loss of functional activity. We subjected particles to full-power sonication (which is not recommended for regular use). Prolonged sonication did not result in measurable loss of biotin binding capacity, as shown in Figure 1, even when the temperature was allowed to rise naturally. Note that in sensitive systems it is important to guard against a rise in temperature during sonication.

When optimizing the following procedure, consider the characteristics of the specific ligand, and adjust the time and handling to ensure ligand activity.

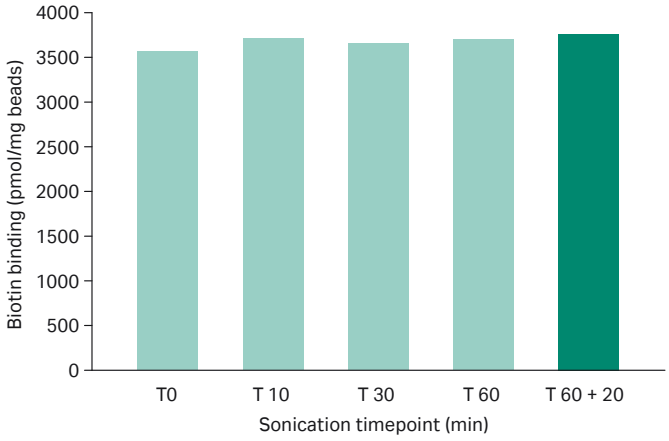


Fig 1. Sera-Mag streptavidin-coated magnetic particles (800 mL in a 1 L container) were sonicated for 1 h. Temperature was controlled using a water bath. Samples taken at 0, 10, 30, and 60 min were assayed for biotin binding capacity. The sample was then sonicated for a further 20 min (T 60 + 20), and the temperature was allowed to rise naturally.

Materials required for sonication

- **Probe sonicator**
  An immersible ultrasonic probe is the recommended tool for efficient resuspension of particle pellets. Vortex mixing and bath-type sonicators are not effective for resuspending most particle pellets.
  Note: Adequate probe maintenance is necessary to ensure proper performance of the sonicator.
- **Appropriate sonicator probe**
  A key factor that affects sonication performance is the choice of sonication probe. Consider the volume to be sonicated when selecting the appropriate probe. For samples ≤ 500 mL or samples in a 1 L narrow-mouth container, we typically use a tapered microtip (1/8 inch diameter). For samples > 500 mL that are not in a narrow-mouth container, we use a large ("macro") tip probe (1/2 inch diameter).
- **Container for sonication**
  If the sample volume is ≤ 1 L, perform sonication in a bottle or transfer it to a beaker. For samples > 1 L in a narrow-mouth container, transfer to an appropriate-size beaker before sonication. Typically, sonication is more effective in a glass container than in a plastic one.
- **Optical microscope and necessary supplies**
  Microscope needs to be capable of 400× magnification.

Sonication procedure

Note: To avoid damaging particles, do not sonicate volumes < 10 mL. Sonicating small sample volumes will heat the solution quickly because the volume is too low to sufficiently disperse the heat. Use vortexing instead of sonication.

1. Thoroughly mix the particles before starting sonication. Use a mechanical roller to mix material in bottles, or use an overhead mixer for bulk material. Follow the mixing procedures in this document.

2. Select the appropriate sonication intensity for the probe. When using the microtip probe, set the intensity between 30% and 40%, or 3 to 4 on a scale of 10. When using the large probe, set the intensity to 50%, or 5 on a scale of 10.

3. Table 1 lists appropriate sonication times for the probe type and volume of material. Perform sonication according to the recommendations, checking for particle monodispersity after a set amount of sonication time.

Table 1. Recommended sonication times

Probe	Volume to be sonicated	Sonication time
Microtip (1/8 inch)	10–50 mL	20–30 s
	50–100 mL	30–45 s
	100 mL to 1 L	> 60–90 s*†
Large (1/2 inch)	1 L	5 min*†
	3 L	5–10 min*
	> 3 L	> 20 min*

* If material in larger samples settles out of solution too quickly, mix it during sonication or sonicate using more repetitions of shorter times.

† If sonicating in a 1 L bottle, roll the bottle for 5 min after every period of sonication.

4. To check for monodispersity, mix the sample thoroughly and observe a portion under a microscope at 400× magnification. If aggregates are visible as in Figure 2A, the material is not monodispersed. Repeat sonication and observation until clumps are not visible. Figure 2B shows an example of a uniform field indicating monodispersed particles.

Mixing overview

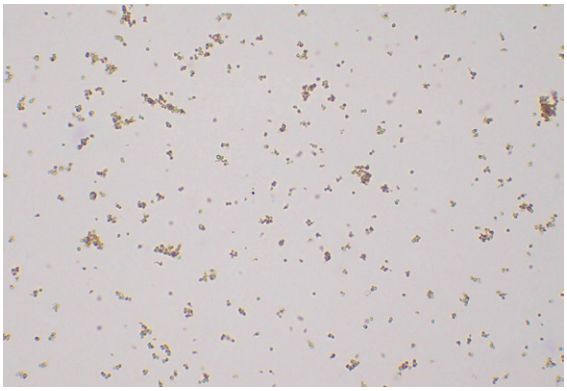
When handling particles, mix the material to ensure it is monodispersed and uniformly distributed. A roller mixer can be used to resuspend particles, if necessary, and to uniformly mix them in a closed container. A vortex mixer can be used for mixing product in small containers such as 15 mL bottles. An overhead mixer is typically used for pooling, diluting, and handling large batches.

Mixing procedures

Considerations for all mixing equipment

- If the sample contains higher than normal levels of surfactant, or if excessive foaming is observed, reduce the speed and time of mixing to minimize damage to the particles.
- When resuspending material, visually confirm (if possible) that resuspension is complete by checking the bottom of the container for unsuspended material.

(A)



(B)

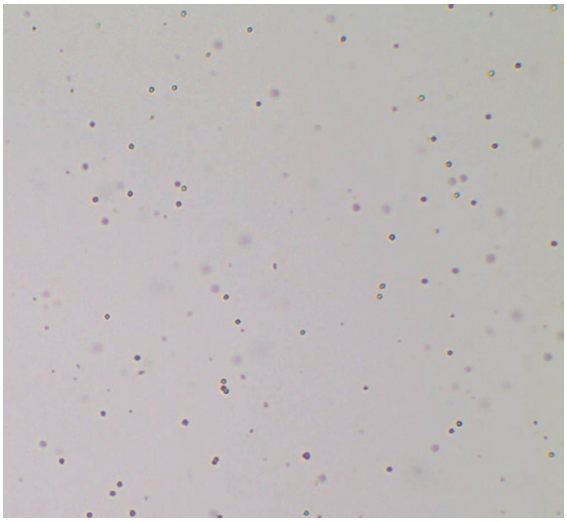


Fig 2. Microscopic examination of particle dispersity after sonication. (A) Severe clumping; (B) Monodispersed particles. 400× magnification.

Using a roller mixer

Overview

The roller mixer has a motor-driven horizontal cylinder adjacent to a free-turning horizontal cylinder that together form a cradle on which containers of product can be placed. The placement of the free-turning cylinder can be adjusted as necessary to accommodate containers of different sizes. Use a roller mixer of sufficient size and speed for the container being mixed. A roller for smaller bottles such as 100 mL can be purchased from small laboratory supply catalogs.

Mixing time

Because the speed of the mixer is constant, mixing time is used to control mixing. The diameter of the container can affect the mixing time; mixing can be accomplished more quickly with small-diameter containers, because they rotate faster than large-diameter ones. Higher concentrations of particles also require more mixing time. The mixing time should be at least 40 min for containers < 1 L and at least 60 min for ≥ 1 L containers. Extending the mixing time is acceptable, up to 72 h.

Container capacity

Containers must be at least 50% and less than 90% full to have enough material covering the bottom of the container when rolling, yet not be too full to prevent insufficient mixing.

Using a vortex mixer

Overview

A vortex mixer is used to mix small volumes (≤ 10 mL). The container of material is placed in a rubber holder, and the motor is allowed to rotate the shaft in an oscillating motion that causes the material to be mixed.

Different vortex mixer models have different methods of activation. Most have both continuous action and manual pressure-activated systems. Either mode may be used, but the continuous mode is preferred for longer vortexing times and the manual mode for shorter mixing times. A vortex mixer with adjustable speed setting is recommended.

Mixing speed

Using the controller on the mixer, adjust to a speed sufficient to cause good mixing (usually around 80% of full speed). A speed that is too fast makes the container difficult to control.

When resuspending Sera-Mag particles, mix for ≥ 1 min, especially if the product has been stored for an extended period of time.

Confirmation of adequate mixing

Observe the product during mixing to ensure adequate agitation. After mixing, make sure no product remains settled on the bottom of the container. Clumps should not be observed in the suspension under a microscope at 400× magnification.

Using an overhead mixer

Overview

An overhead mixer has an electrical or air-driven motor with an agitator blade and shaft attached. The speed is controllable. Choose an overhead mixer and agitator that is sufficient to mix the required volume of material.

Volume and container

It is important to use an agitator that is appropriate for the volume to be mixed. We recommend using a short-shafted agitator for smaller volumes.

- Use a container that allows the agitator blade to be covered with enough product to prevent splashing,
- Position the blade high enough on a stand to allow clearance of the container (but not so high that the agitator will not be sufficiently submerged). Best results are usually obtained when the agitator blade can be placed at a position in the lower one-third of the container.

Mixing speed

The proper mixing speed can be determined by observing the material in the container. If there is no visible movement of the product, increase the speed until movement is seen.

In most circumstances, overhead stirring is used to achieve or maintain a uniform mixture. Therefore, mixing speed is not critical as long as sufficient motion is maintained.

If aliquots will be removed from the mixture, carefully monitor the level of product being mixed and periodically reduce the speed of the mixing to keep the product from splashing on the side of the container as the volume changes.

Mixing time

If mixing is for resuspension, mix for at least 40 min for containers < 1 L and at least 60 min for larger containers.

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