

Sera-Mag™ Oligo(dT)-coated magnetic particles

Instructions for Use

1 Introduction

Important

Read the *Instructions for Use* carefully before using the products.

Intended use

The products are intended for research use only, and must not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Storage

Store the products at 2°C to 8°C.



NOTICE

Do not freeze the product.

2 Safety precautions

Definitions

This user documentation contains safety notices (CAUTION and NOTICE) concerning the safe use of the product. See definitions below.



CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.



NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

General precautions

To handle the product in a safe way, follow the precautions below. For more information refer to the Safety Data Sheet.



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.



CAUTION

Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used.

3 Background

Description

Sera-Mag™ Oligo(dT)-coated magnetic particles are nominal 1 µm, superparamagnetic particles of uniform size. The particles are coated with oligo(dT) designed to capture or isolate mRNA from a variety of sources.

Sera-Mag Oligo(dT)-coated magnetic particles are referred as "the particles" or "the magnetic particles" interchangeably in this document.

Surface characteristics

Sera-Mag Oligo(dT)-coated magnetic particles are coated with oligo(dT), which has a high poly A+ binding capacity. This feature supports isolation of intact mRNA from eukaryotic total RNA or directly from crude extracts of cells, animal, and plant tissues.

Specifications

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

Typical applications

Sera-Mag Oligo(dT)-coated magnetic particles isolate and extract mRNA from a variety of sources. The isolated mRNA can be used for many molecular biology applications including:

- RT-PCR cDNA library construction
- RT-qPCR
- NGS, expression analysis
- cDNA library construction
- cDNA microarrays
- primer extension
- subtractive hybridization

Visit our application library <https://www.cytivalifesciences.com/en/us/solutions/genomics> for a list of published articles referencing the Sera-Mag Oligo(dT)-coated magnetic particles.

4 Preparation

Reagent preparation

To prepare the reagents follow the guidelines described in the protocol you are following, and the manufacturer's recommendations for reagent preparation and use.

To prepare reagents for the protocol for isolation of mRNA from total RNA, see [Recommended reagents, on page 7](#).

To prepare reagents for the protocol for isolation of mRNA from crude lysates, see [Recommended reagents, on page 14](#).

For best results, allow the binding and wash buffers to equilibrate to ambient temperature before use. If precipitate is observed in any of the buffers, warm the buffer at ambient temperature and mix until the precipitate is completely dissolved.

The Sera-Mag Oligo(dT)-coated magnetic particles should be thoroughly resuspended by vortexing before use. The particles can be placed on a roller mixer at room temperature (RT) for 30 minutes before use to make sure the contents are thoroughly mixed.

5 Isolation of mRNA from total RNA

Introduction

The following procedure describes the isolation of mRNA from total RNA using Sera-Mag Oligo(dT)-coated magnetic particles.

5.1 Recommendations

Important

This procedure is recommended as a starting point for mRNA isolation from total RNA and might require further optimization.

Recommended equipment

The following equipment is recommended to use in this protocol:

- 0.2 mL tubes (low-binding, sterile, DNase and RNase-free).
- Single-channel pipette (when processing one sample) or multi-channel pipette (when processing more than one sample).
- Thermal cycler or heating block, suitable for use with 0.2 mL tubes.
- Centrifuge, suitable for use with 0.2 mL tubes.
- Magnetic separation rack, suitable for use with 0.2 mL tubes.

Note: *All materials, tubes, and pipette tips should be graded RNase-free. The working environment should be cleaned to minimize likelihood of contamination with extraneous DNase, RNase, and DNA/RNA material.*

Recommended input volume

This protocol has been optimized for a sample input ranging from 250 to 1000 ng total RNA.

Recommended reagents

The following table lists the reagents that are recommended when isolating mRNA from total RNA.

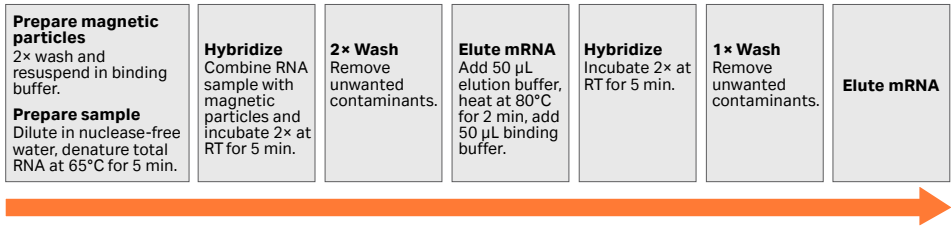
Note: All reagents should be RNase-free.

Reagent	Composition
Binding buffer	40 mM Tris-HCl (pH 7.5) 1 M lithium chloride (LiCl) 2 mM EDTA 0.1% Triton X-100
Wash buffer	20 mM Tris-HCl (pH 7.5) 150 mM LiCl 1 mM EDTA 0.01% Triton X-100
Elution buffer	10 mM Tris-HCl (pH 7.5)
Nuclease-free water	

Note: Buffer compositions are intended as a guide only and might require further optimization.

5.2 Isolation protocol

The protocol for isolation of mRNA from total RNA follows the workflow below.



Prepare the magnetic particles

Follow the steps below to wash and resuspend the Sera-Mag Oligo(dT)-coated magnetic particles.

Allow binding buffer to equilibrate at room temperature. See [Reagent preparation, on page 5](#) for more information.

Step	Action
1	Resuspend the Sera-Mag Oligo(dT)-coated magnetic particles thoroughly by vortexing.
2	Aliquot 10 µL of particles into a sterile, RNase-free tube. Note: <i>It is recommended to use 0.2 mL tubes.</i>
3	Add 100 µL of binding buffer to the particles. Mix by pipetting up and down 6 times.
4	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
5	Remove and discard the supernatant. Note: <i>Take care to not disturb the particles.</i>
6	Remove the tube from the magnetic rack.
7	Repeat steps 3 to 6.
8	Resuspend the particles in 50 µL of binding buffer.
9	Mix by pipetting up and down until particles are homogenous.

Leave the particles in binding buffer until the first hybridization step is started.

Prepare the sample

Follow the steps below to prepare the total RNA sample for binding to the magnetic particles.

Step	Action
1	Dilute the total RNA sample with nuclease-free water to a final volume of 50 µL in a sterile, RNase-free tube. Keep on ice. Note: <i>It is recommended to use 0.2 mL tubes.</i>
2	Mix the diluted sample thoroughly by pipetting up and down 6 times.

Step	Action
3	Using a thermal cycler or a heating block, heat the sample for 5 minutes at 65°C and then cool to 4°C.
	Note: <i>If using a thermal cycler, set the heated lid to $\geq 75^{\circ}\text{C}$. Use of a heated lid is optional when using a heating block.</i>
4	Remove the tube from the instrument once the temperature reaches 4°C and place it on ice.

Keep the sample on ice until the first hybridization step is started.

Hybridize mRNA to particles

Follow the steps below to bind the magnetic particles to the mRNA in your sample.

Step	Action
1	Add 50 μL of the prepared Sera-Mag Oligo(dT)-coated magnetic particles (see Prepare the magnetic particles, on page 7) to the prepared RNA sample (see Prepare the sample, on page 8).
2	Mix thoroughly by pipetting up and down six times.
3	Incubate the tube at room temperature for 5 minutes.
4	Resuspend the particles by slowly pipetting up and down six times.
5	Incubate the tube at room temperature for 5 more minutes.
6	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
7	Remove and discard all of the supernatant.
	Note: <i>Take care not to disturb the particles.</i>
8	Remove the tube from the magnetic rack.

Wash the hybridized particles

Follow the steps below to wash the magnetic particles to remove unwanted contaminants.

Allow wash buffer to equilibrate at room temperature. See [Reagent preparation, on page 5](#) for more information.

Step	Action
1	Add 200 µL of wash buffer to the sample tube (see Hybridize mRNA to particles, on page 9).
2	Mix thoroughly by pipetting the entire volume up and down 6 times.
3	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
4	Remove and discard all the supernatant from the tube.
	Note: <i>Take care not to disturb the particles.</i>
5	Remove the tube from the magnetic rack.
6	Repeat steps 1 to 5.

Elute the mRNA

Follow the steps below to perform the first elution of the mRNA from the magnetic particles.

Step	Action
1	Add 50 µL of elution buffer to the sample tube.
2	Gently pipette the entire volume up and down 6 times to mix thoroughly.
3	Using a thermal cycler or a heating block, heat the sample for 2 minutes at 80°C and then cool to 25°C.
	Note: <i>If using a thermal cycler, set the heated lid to ≥90°C. Use of a heated lid is optional when using a heating block.</i>
4	Remove tubes from the instrument once the temperature reaches 25°C.

Re-hybridize mRNA to particles

Follow the steps below to perform the final hybridization step.

Step	Action
1	Add 50 µL of binding buffer to the sample tube (see Elute the mRNA, on page 10).

Step	Action
2	Mix thoroughly by pipetting up and down six times.
3	Incubate the tube at room temperature for 5 minutes.
4	Resuspend the particles by pipetting up and down six times.
5	Incubate the tube at room temperature for 5 more minutes.
6	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
7	Remove and discard all of the supernatant.
	Note: <i>Take care not to disturb the particles.</i>
8	Remove the tube from the magnetic rack.

Wash the re-hybridized particles

Follow the steps below to wash the magnetic particles to remove unwanted contaminants.

Step	Action
1	Add 200 μ L of wash buffer to the sample tube (see Re-hybridize mRNA to particles, on page 10).
2	Mix thoroughly by gently pipetting the entire volume up and down 6 times.
3	Spin down the tube briefly.
4	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
5	Remove and discard all the supernatant from the tube.
	Note: <i>Make sure to remove all of the supernatant thoroughly in this step. Repeat steps 3 to 5 and use a 10 μL pipette tip in step 5 to make sure all wash buffer is removed.</i>
	Note: <i>Take care not to disturb the particles.</i>
	Note: <i>Do not let the particles dry out.</i>

Step	Action
6	Remove the tube from the magnetic rack.

Elute the purified mRNA

Follow the steps below to elute the purified mRNA sample.

Step	Action
1	Add 17 μ L of elution buffer to the sample tube.
2	Pipette the entire volume up and down 6 times to mix thoroughly.
3	Using a thermal cycler or a heating block, heat the sample for 2 minutes at 80°C and then cool to 25°C.
	Note: <i>If using a thermal cycler, set the heated lid to $\geq 90^{\circ}\text{C}$. Use of a heated lid is optional when using a heating block.</i>
4	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
5	Transfer 15 μ L of the supernatant containing the purified mRNA to a clean, sterile RNase-free tube.

Store on ice for immediate quantitation. If the isolated mRNA is not used immediately, store at -80°C .

6 Isolation of mRNA from crude lysates

Introduction

The following procedure describes the isolation of mRNA from crude lysates using Sera-Mag Oligo(dT)-coated magnetic particles. This protocol is recommended for mRNA purification from mammalian cultured cells and cell suspensions.

Note: *It is possible this protocol could be adapted for use with solid plant or animal tissue, but these sample types have not been tested.*

6.1 Recommendations

Important

This procedure is recommended as a starting point for mRNA isolation from crude lysates and might require further optimization.

Input volume considerations

Magnetic particle to sample ratios can vary depending on sample type.

When an adequate magnetic particle to sample ratio is used, there is no bias in hybridization that is based on mRNA size.

With a large excess of mRNA or short incubation time, binding might be biased towards shorter molecules. A similar bias can occur if the sample volume to magnetic particle ratio is too high.

Recommended input volume

This protocol has been optimized for a sample input ranging from 1×10^4 to 1×10^6 cells. The following table lists recommended volumes of reagents depending on the number of cells.

Note: *The information on the table is intended as a guide only.*

Number of cells	Volume (µL)				
	Sera-Mag Oligo(dT)-coated magnetic particles	Binding buffer	Wash buffer A	Wash buffer B	Elution buffer
1×10^4 to 5×10^4	25	250	250	250	50 ¹
5×10^4 to 1×10^6	50	500	500	500	100

¹ If this volume is insufficient to pull magnetic particles efficiently, increase the elution volume to 100 µL

Recommended equipment

The following equipment is recommended to use in this protocol:

- 1.5 mL or 2.0 mL tubes (low-binding, sterile, DNase and RNase-free).
- Water bath or heating block, suitable for use with 1.5 mL or 2.0 mL tubes.
- Sample mixer.
- Benchtop centrifuges, suitable for use with 1.5 mL, 2.0 mL, and 15 mL tubes.
- Magnetic separation rack, suitable for use with 1.5 mL or 2.0 mL tubes.
- Syringe and 21-gauge needle.

Note: All materials, tubes, and pipette tips should be graded RNase-free. The working environment should be cleaned to minimize likelihood of contamination with extraneous DNase, RNase, and DNA/RNA material.

Recommended reagents

The following table lists the reagents that are recommended when isolating mRNA from crude lysates.

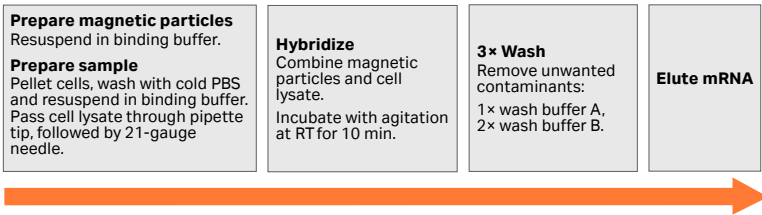
Note: All reagents should be graded RNase-free.

Reagent	Composition
Binding buffer	100 mM Tris-HCl (pH 7.5) 500 mM lithium chloride (LiCl) 10 mM EDTA 1% lithium dodecyl sulfate (LiDS) 5 mM dithiothreitol (DTT)
Wash buffer A	10 mM Tris-HCl (pH 7.5) 150 mM LiCl 1 mM EDTA 0.1% LiDS
Wash buffer B	10 mM Tris-HCl (pH 7.5) 150 mM LiCl 1 mM EDTA
Elution buffer	10 mM Tris-HCl (pH 7.5)
1× PBS (pH 7.4)	

Note: Buffer compositions are intended as a guide only and might require further optimization.

6.2 Isolation protocol

The protocol for isolation of mRNA from crude lysate follows the workflow below.



Prepare the magnetic particles

Follow the steps below to resuspend the Sera-Mag Oligo(dT)-coated magnetic particles. Allow binding buffer to equilibrate at room temperature. See [Reagent preparation, on page 5](#) for more information.

Step	Action
1	Resuspend the Sera-Mag Oligo(dT)-coated magnetic particles thoroughly by vortexing.
2	Aliquot the applicable volume of particles according to the table in Recommended input volume, on page 13 .
3	Add 200 µL of binding buffer to the particles. Vortex to resuspend.
4	Mix with gentle agitation (approximately 300 rpm) for 2 minutes.

Leave the particles in binding buffer until the hybridization step is started.

Prepare the sample

Follow the steps below to prepare the cell lysate sample for binding to the magnetic particles.

Step	Action
1	Centrifuge the applicable number of cells at 400 × g for 5 minutes at 4°C. For more information, see Recommended input volume, on page 13 .
2	Aspirate the media without disturbing the cell pellet.
3	Wash the cell pellet with cold, sterile 1× PBS (pH 7.4).

Step	Action
4	Centrifuge again at $400 \times g$ for 5 minutes at 4°C and discard the supernatant.
5	Add the applicable volume of binding buffer to the cell pellet (according to the table in Recommended input volume, on page 13).
6	Incubate for 5 minutes at room temperature with gentle agitation (approximately 300 rpm).
7	Perform repeated passage of the lysate through a pipette tip.
8	Perform repeated passage of the lysate for 3 to 5 times with a 21-gauge needle attached to a syringe of applicable size.
	Note: <i>Repeated shearing during steps 7 and 8 can result in foaming of the lysate. While foaming should not affect mRNA yield, the sample can be centrifuged briefly to reduce the foam if required.</i> Result: If the initial solution was viscous, a noticeable decrease in viscosity should be observed.

Hybridize mRNA to particles

Follow the steps below to bind the magnetic particles to the mRNA in your sample.

Step	Action
1	Place the prepared Sera-Mag Oligo(dT)-coated magnetic particles (see Prepare the magnetic particles, on page 15) into the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
2	Remove and discard the supernatant.
3	Add the prepared cell lysate sample (see Prepare the sample, on page 15) to the tube.
4	Incubate the tube for 10 minutes at room temperature with gentle agitation (approximately 300 rpm).
5	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
6	Remove and discard the supernatant.
7	Remove the tube from the magnetic rack.

Wash the hybridized particles

Follow the steps below to wash the magnetic particles to remove unwanted contaminants.

Repeat the steps with the reagent specified in the table for each of the three wash rounds.

Round	Reagent
Wash 1	Wash buffer A
Wash 2	Wash buffer B
Wash 3	Wash buffer B

Allow wash buffers to equilibrate at room temperature. See [Reagent preparation, on page 5](#) for more information.

Step	Action
1	Add the recommended volume of the applicable wash buffer (according to the table in Recommended input volume, on page 13) to the sample tube (see Hybridize mRNA to particles, on page 16).
2	Vortex briefly to resuspend and mix with gentle agitation (approximately 300 rpm) for 1 minute at room temperature. Note: <i>Make sure to thoroughly resuspend magnetic particles to avoid salt and detergent carryover.</i>
3	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
4	Remove and discard the supernatant. Note: <i>Make sure to completely remove the supernatant to avoid salt and detergent carryover.</i>
5	Remove the tube from the magnetic rack.
6	Repeat steps 1-5 two more times, for a total of 3 times per wash round.

Elute the purified mRNA

Follow the steps below to elute the purified mRNA sample.

Step	Action
1	Add the recommended volume of elution buffer (according to the table in Recommended input volume, on page 13) to the sample tube (see Wash the hybridized particles, on page 17).
2	Vortex gently to resuspend the particles.
3	Incubate at 50°C for 2 minutes with occasional gentle agitation (approximately 300 rpm).
4	Place the tube in the magnetic rack until the particles have been pulled to the side of the tube and the solution is clear.
5	Transfer the supernatant containing purified mRNA to a clean, sterile RNase-free tube.

Store on ice for immediate quantitation. If the isolated mRNA is not used immediately, store at -80°C.

7 Troubleshooting

Below you can find guidance on how to solve common issues. If problems persist and for further information, contact Cytiva scientific support.

For contact information, see <http://cytiva.com/contact>.

Problem	Possible Cause	Corrective action
mRNA yield is low	RNA degradation	Make sure all reagents and consumables are RNase-free. Keep samples on ice wherever possible. The starting material (total RNA or cells) must be of high quality. Make sure it has been stored and handled appropriately to prevent any RNA degradation prior to processing. Store eluted mRNA samples at the correct temperature to avoid degradation: • For short-term storage, store purified mRNA at –20°C. • For longer term storage, store mRNA at –80°C. • Store in single-use aliquots to prevent damage to the mRNA from multiple freeze-thaw cycles and prevent RNase contamination. If applicable, RNase inhibitors can be used throughout the protocol or in the eluted mRNA sample.
Non-specific binding	Ribosomal RNA (rRNA) contamination	Use of more stringent wash buffers can help reduce rRNA contamination. Optimization might be required. Note: <i>For an optimized custom buffer, contact your Cytiva sales representative for pricing and availability.</i> Perform additional washes during the isolation process to remove any non-specifically bound rRNA.

Problem	Possible Cause	Corrective action
	Genomic DNA (gDNA) contamination	When isolating mRNA from crude lysates: • If genomic DNA is a concern, the lysate can be treated with DNase I prior to the isolation process. • If the cell lysate remains viscous after shearing, possible solutions are to lower the ratio of cells to binding buffer volume and to increase the binding incubation time. • Non-sheared gDNA can cause clumping of the magnetic particles. If clumping is observed, rapidly pipette cell lysate up and down with a pipette to reduce viscosity. It is possible to isolate mRNA under these conditions, but it is more likely to be contaminated with gDNA.

8 Ordering information

For additional information, refer to [cytiva.com](https://www.cytiva.com).

Products

Product	Pack size	Product code
Sera-Mag Oligo(dT)-coated magnetic particles	1 mL	38152103011150
	5 mL	38152103010150
	100 mL	38152103010350



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