Technical Note:
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Thermo Scientific Sera-Mag Oligo(dT)-Coated Magnetic Particles Procedure

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Key Words:

- Magnetic Particles
- Oligo(dT)
- mRNA Purification
- Poly A RNA
- Hybridization (Binding) Buffer
- Wash Buffer
- Elution Buffer

Thermo Scientific complete particle technology solutions provide simple protocols for working with particles and concrete data backed by 30 years of proprietary applications research in our labs.

1. INTRODUCTION

Thermo Scientific Sera-Mag Oligo(dT)-coated magnetic particles (MG-OL) easily isolate and extract valuable mRNA from a variety of sources for use in such applications as RT-PCR, cDNA library construction, cDNA microarrays, affinity purification, primer extension and subtractive hybridization.

Combined with any generic buffer, Sera-Mag Oligo(dT)-coated magnetic particles (Cat. No. 3815-2103-011150, 1 mL) provide a convenient and rapid method for isolating high purity mRNA from total RNA samples.

Sera-Mag particles also provide the high surface area, high affinity and high specific activity required to deliver high purity mRNA. By using a magnetic particle format, the process can be easily scaled up or down according to the sample, providing the scalability and flexibility for downstream applications.

In addition to being colloidally stable in the absence of a magnetic field, these particles can be separated rapidly and completely from suspension when a magnetic field is applied.

Binding of polyadenylated RNA (poly-A+ RNA) to the covalently bound oligo (dT) groups on the surface is easily accomplished using standard hybridization conditions.

Other RNA species (rRNA and tRNA) do not contain poly A+ sequences and therefore will not bind to the oligo(dT) particles. As a result, the isolated mRNA can be directly used in cloning and expression analysis applications.

The oligo(dT) particles can also be used as a universal base particle to attach a unique oligo sequence. By synthesizing a poly A+ tail on your oligo, you can easily bind this to the dT surface of the particles to create a uniquely coated magnetic particle.

2. PRODUCT DESCRIPTION

Sera-Mag Oligo(dT)-coated magnetic particles are uniform, colloidally stable, monodisperse, non-porous superparamagnetic spheres made by a proprietary core-shell method.

The core is a carboxylate-modified particle made by free radical emulsion polymerization of styrene and acid monomer.

Magnetite (Fe_3O_4) is coated onto this core particle and then encapsulated with proprietary polymers.

These stable, nominal 1 µm particles have a highly active surface with covalently bound oligo$(dT)_{14}$, and are supplied at approximately 1% solids (10 mg/mL) in 0.05 % sodium azide.

All Thermo Scientific particles are manufactured under strict quality and QSR controls in our medical device registered, ISO-13485 certified facility.

3. IMPORTANT PRODUCT INFORMATION

Before You Begin

- Prepare all required materials before starting in order to minimize RNA degradation
- When working with RNA, always wear gloves to minimize RNase contamination
- Change gloves frequently
- Use only clean RNase-free disposable plastic pipette tips when using the buffers
- When possible, perform mRNA extractions from isolated total RNA
- Hybridize at temperatures from room temperature up to 40°C. Hybridization takes place within 5 to 15 minutes.

4. USING THE THERMO SCIENTIFIC SERA-MAG OLIGO(dT) BUFFER KIT WITH SERA-MAG OLIGO(dT)-COATED MAGNETIC PARTICLES

The following, modifiable protocol provides a tool for evaluating Thermo Scientific Sera-Mag Oligo(dT)-coated magnetic particles.

Materials Required

- Sera-Mag Oligo(dT)-coated magnetic particles (Cat. No. 3815-2103-011150, 1 mL)
- Thermo Scientific Buffer Kit (Cat. No. 281111)
  - Hydridization, wash and elution buffers included in kit

Note: Any other buffer set of choice may be used.

- Nuclease-free 1.5 mL microcentrifuge tubes
- Magnetic stand for 1.5 mL tube
- Absolute ethanol, chilled to -20°C
- Microcentrifuge

Note: Exact mRNA isolation applications information has not been fully developed for these particles. Use these general recommendations when working with Sera-Mag Oligo(dT)-Coated particles and then modify to your specific application conditions.

Standard Evaluation Protocol Using Magnetic Separator Stand

This protocol is for the purification of mRNA from 100 µg total RNA. Reactions may be scaled down to 0.5x for 50 µg total RNA, or up to 10x for up to 1 mg total RNA, all in a single reaction tube. When scaling down or up, decrease or increase the volume of all components, including the oligo(dT)-coated particles.

1. Prepare the total RNA (100 µg) in 100 µL of mRNA elution buffer or 100 µL of RNase-free water.

   Note: If the total RNA is more diluted than 1 µg/µL, the 100 µg of RNA may be added in a larger sample volume. In this case, increase the volume of mRNA hybridization buffer used in Step 4 to equal the initial volume of the total RNA sample.

2. Heat the total RNA solution to 65°C for 4 minutes, and then transfer the sample to ice.

3. Sonicate, vortex or roll the vial of oligo(dT) coated magnetic particles to ensure that they are in a homogenous suspension.

   Transfer 50 µL of the particle suspension to an RNase-free microcentrifuge tube. Wash the particles twice with 100 µL of mRNA hybridization buffer.

   During each wash, mix by pipetting and then collect the particles using a magnetic separator stand, in accordance to the manufacturer's instructions.

   Note: The hybridization buffer may precipitate when stored at cooler temperatures. Store the buffer at room temperature and do not place the buffer on ice. If a precipitate is present, warm the buffer to 50°C and swirl the solution to redissolve the components.

   Whenever the particles are collected using the magnetic separator, leave the tube in the magnetic stand while removing the supernatant.

4. Resuspend the washed particles in 100 µL of mRNA hybridization buffer by repeated pipetting.

5. Add 100 µL of the total RNA solution to the resuspended magnetic particles. Mix by repeated pipetting.

6. Incubate the mixture at room temperature with gentle agitation for 5 minutes to allow hybridization of the mRNA to the particles.

7. Collect the magnetic particles complexed with mRNA using a magnetic separator stand.

8. Remove the supernatant with a pipette tip.

   Note: The supernatant, containing the unbound fraction of the total RNA may be retained, if desired, to facilitate troubleshooting or may be discarded at this step.

9. Wash the mRNA-bound magnetic particles three times using 100 µL of mRNA wash buffer for each wash. Resuspend the particles during each wash, and then recollect them using the magnetic separator stand.

   Be sure to remove all of the wash buffer when completing the final wash.

10. Remove the tube from the magnetic stand and then add 100 µL of mRNA elution buffer to the particles. Incubate the tube at room temperature with gentle agitation for 5 minutes to release the mRNA from the particles.

11. Collect the magnetic particles using a magnetic separator stand.

12. Draw off the 100 µL eluate containing the purified mRNA, and transfer the solution to a fresh, RNase-free tube. The RNA can be stored at -20°C for up to one month or at -80°C for long-term storage.

Expected RNA yields and quantification

To quantify mRNA isolated from 50 µg to 1 mg total RNA, a highly sensitive fluorescence-based system (e.g., RiboGreen® RNA quantitation kit, Invitrogen, Inc.) may be used.

The expected yield of mRNA is 1-5% of the amount of total RNA starting material.

Appendix: Alternate purification protocol using particle centrifugation

The protocol below is for the purification of mRNA from 100 µg total RNA. Reactions may be scaled down to 0.5x for 50 µg total RNA, or up to 10x for up to 1 mg total RNA, all in a single reaction tube.

When scaling down or up, simply decrease or increase the volumes of all components, including the oligo(dT) coated magnetic particles.

1. Prepare the total RNA (100 µg) in 100 µL of mRNA elution buffer or 100 µL of RNase-free water.

   Note: If the total RNA is more dilute than 1 µg/µL, the 100 µg of RNA may be added in a larger sample volume. In this case, increase the volume of mRNA hybridization buffer used in Step 4 to equal the initial volume of the total RNA sample.

2. Heat the total RNA solution of 65°C for 4 minutes, and then transfer the sample to ice.

3. Sonicate, vortex or roll the vial of Sera-Mag Oligo(dT)-coated magnetic particles to ensure that they are in a homogenous suspension. Transfer 50 µL of the particle suspension to an RNase-free microcentrifuge tube. Wash the particles twice with 100 µL of mRNA hybridization buffer.

   During each wash, mix by pipetting and then collect the particles by centrifugation at 10,000 x g for 2 minutes.

   Note: The hybridization buffer may precipitate when stored at cooler temperatures. Store the buffer at room temperature and do not place the buffer on ice. If a precipitate is present, warm the buffer to 50°C and swirl the solution to redissolve the components.

   Whenever the particles are collected by centrifugation, remove the supernatant carefully to avoid disturbing the pelleted particles.

4. Resuspend the washed particles in 100 µL of mRNA hybridization buffer by repeated pipetting.

5. Add 100 µL of the total RNA solution to the resuspended magnetic particles. Mix by repeated pipetting.

6. Incubate the mixture at room temperature with gentle agitation for 5 minutes to allow hybridization of the mRNA to the particles.

7. Collect the magnetic particles complexed with mRNA by centrifugation at 10,000 x g for 2 minutes.

8. Remove the supernatant with a pipette tip.

   Note: The supernatant, containing the unbound fraction of the total RNA may be retained, if desired, to facilitate troubleshooting or may be discarded at this step.

9. Wash the mRNA-bound magnetic particles three times using 100 µL of mRNA wash buffer for each wash.

   Resuspend the particles during each wash, and then recollect them by centrifugation at 10,000 x g for 2 minutes.

   Be sure to remove all of the wash buffer when completing the final wash.

10. Add 100 µL of mRNA elution buffer to the particles. Incubate the tube at room temperature with gentle agitation for 5 minutes to release the mRNA from the particles.

11. Collect the magnetic particles by centrifugation at 10,000 x g for 2 minutes.

12. Draw off the 100 µL eluate containing the purified mRNA, and transfer the solution to a fresh, RNase-free tube. The RNA can be stored at -20°C for up to one month or at –80°C for long-term storage.

5. TROUBLESHOOTING

General troubleshooting tips and suggestions for Sera-Mag Oligo(dT)-coated magnetic particles are shown below.

Problem	Possible Cause	Solution
Degraded RNA	RNase contamination from handling	Follow exact protocol and work quickly Wear gloves throughout the procedure and when handling the solution and equipment used for RNA isolation. Do not introduce RNase during the procedure
	RNase contamination from total RNA sample	Check total RNA sample for RNase contamination: Analyze sample by agarose gel electrophoresis. RNase contamination can be determined by loss or smear of 18S and 28S rRNA bands.
rRNA contamination	rRNA co-purified with mRNA	Ensure total RNA samples are heated at 65°C prior to addition of magnetic particles. If the rRNA level is too high for downstream applications, purify the mRNA with a second round of purification with new magnetic particles
mRNA does not elute	Elution conditions are too mild	Increase incubation time with elution buffer or use more stringent elution buffer
Magnetic particles aggregate	Magnetic particles were frozen or centrifuged	Handle the particles as directed in the instructions
	Buffer used is incompatible with magnetic particles	

6. ADDITIONAL INFORMATION

Visit www.thermoscientific.com/particletechnology for additional information on this product.

In addition to our office, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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