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Revised June 2007

Introduction

High purity mRNA is critical for downstream applications such as RT-PCR and QRT-PCR, The E.Z.N.A.[®] Mag-Bind mRNA Purification Kit provides a convenient and rapid method for the isolation of high purity of mRNA from tissue and total RNA samples. This kit is based on Mag-Bind magnetic particles which have a large surface compare to other standard magnetic beads and delivery high purity of mRNA. The magnetic bead format also can be easily scaled up and down according to the sample, offering scalability and flexibility for a variety of downstream applications.

If using the E.Z.N.A.[®] Mag-Bind mRNA Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. The sample is lysed first under highly denaturing buffer conditions so that RNases will be inactivated, and the intact tissue RNA is protected from degrading. Total cellular RNA is precipitated with isopropanol and resuspend. The oligo(dT) magnetic particles are mixed with total RNA solution. Poly(A)+ RNA hybridizes to the magnetic particles under optimized conditions. After apply the magnetic field, the magnetic particle/mRNA complexes is pulled out of the solution. Contaminants are removed by aspiration, and then the magnetic beads are thoroughly washed by two quick wash steps. Purified mRNA is eluted from magnetic particles in an aqueous solution.

Storage and Stability

All components of the E.Z.N.A.[®] Mag-Bind mRNA Kit should be stored at 22°C-25°C. Under these conditions, RNA has successfully been purified and used for RT-PCR after 24 months of storage. **Do not frozen the Mag-Bind oligo(dT) magnetic beads solution .**

Binding Capacity

100ul of the **Mag-Bind oligo(dT) magnetic beads solution** can bind approximately 1.5µg mRNA.

Kit Contents

Product No.	R6570-00	R6570-01	R6570-02
Purification	2	10	30
Oligo(dT) magnetic beads	110 µl	550µl	1.5 ml
RNA-Solv® Reagent II	3 ml	6 ml	16 ml
2 x Mag-Bind mRNA Binding	500 µl	5 ml	15 ml
Mag-Bind mRNA Wash Buffer	500 µl	3 ml	10 ml
mRNA Elution Buffer	500 µl	1.5 ml	5 ml
User Manual	1	1	1

Before Starting

IMPORTANT	Add water-saturated phenol to RNA-Solv® Reagent II before use:
	R6570-00 Add 4.5ml water-saturated phenol per bottle
	R6570-01 Add 9ml water-saturated phenol per bottle
	R6570-02 Add 24ml water-saturated phenol per bottle

Prepare water-saturated phenol:

1. Dissolve solid phenol in a 65 °C water bath.
2. Add equal volume of ddH₂O and shake to mix thoroughly.
3. Leave for overnight to completely saturate the phenol.
4. Remove the top water phase with transfer pipette.
5. The phenol is ready to use.

Working with RNA

Please take a few minutes to read this booklet thoroughly to become familiar with the protocol. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Change gloves frequently. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- During the procedure work carefully but quickly.
- Under cool ambient conditions, crystals may form in Mag-Bind mRNA Binding Buffer. This is normal and the bottle may be warmed to 50 °C to redissolve the salt.

Mag-Bind mRNA Protocol (Standard Protocol)

Materials to be provided by user

- Magnetic Stand for 1.5 ml tube (OBI # MSTD-02)
- Nuclease-free 1.5ml centrifuge tubes
- Absolute Ethanol, chilled at -20°C
- Nuclease-free 15 ml plastic conical centrifuge tubes
- Microcentrifuge
- Chloroform

This protocol is for isolating mRNA from up to **100 mg** tissue or **5 x 10⁶ cells**. (Scale-up accordingly for larger samples) When scale up or down, simply increase or decrease the volume of all reagents include the Mag-Bind oligo(dT) magnetic beads.

1. **Homogenization and lysis of samples:** follow either method below.

a) Tissue Samples

Homogenize tissue samples in 1 mL of RNA-Solv® Reagent per 50-100 mg of tissue using an appropriate mechanical homogenizer. Alternatively one can pulverize tissue in liquid nitrogen with mortar and pestle and transfer the powder to a clean 1.5 ml microcentrifuge tube. If ceramic mortar and pestle are not available, homogenize the sample in the microfuge tube using a disposable microtube pestle (OBI Cat No. SS-1015). The sample volume should not exceed 10% of the volume of RNA-Solv® Reagent used.

b) Cells Grown in Suspension

Pellet cells by centrifugation. Lyse cells in RNA-Solv® Reagent by repetitive pipetting. Use 1 mL of the reagent per 5- x 10⁶ of animal, plant or yeast cells, or per 1 x 10⁸ bacterial cells. Washing cells before addition of RNA-Solv® Reagent should be avoided as this increases the possibility of mRNA degradation and RNase contamination

c) Cells Grown in Monolayer

Lyse cells directly in a culture dish by adding 1 mL of RNA-Solv® Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a blue pipette tip. The amount of RNA-Solv® Reagent added is based on the area of the culture dish (~1 mL per 10 cm²). An insufficient amount of RNA-Solv® Reagent may result in contamination of the isolated RNA with DNA. Always use more RNA-Solv® Reagent if the lysate is too viscous to aspirate with a pipette.

Note: Add water-saturated phenol to RNA-Solv Reagent II before use.

2. Add 0.2 mL of chloroform per 1 mL of RNA-Solv® Reagent II/phenol solution.

Cap sample tubes securely and vortex vigorously for 15 seconds. Incubate on ice for 10 minutes. *This step is critical - do not change it.*

3. Centrifuge at 13,000 x g for 5 minutes at 4° C.
4. **Precipitation of RNA.** Transfer no more than 80% of the aqueous phase to a fresh tube, and discard the lower organic phase. Precipitate the RNA from the aqueous phase by adding 500 µl of isopropyl alcohol per 1 mL of RNA-So_lv® Reagent used for the initial homogenization. Incubate samples at room temperature 10 minutes and centrifuge at no more than 12,000 xg for 10 minutes also at room temperature.
5. Collect the supernatant (containing RNA) with a fine tip pipette and transfer to a conical centrifuge tube. Measure the volume and add ½ volume isopropanol (For 1 ml RNA-So_lv® Reagent II/phenol solution, add 500µl isopropanol). Vortex well to mix and incubate the sample at room temperature for at least 10 minutes.
6. Carefully aspirate and discard the isopropanol and briefly **AIR DRY** the RNA pellet for 5-10 minutes at room temperature. Do not use centrifugal devices equipped with a vacuum source as over-drying will lead to difficulty in re-dissolving RNA in water.
7. Add 100µl RNase-free water or 0.5% SDS solution and vortex for 20 seconds. Incubate at 55°C for 10 minutes to completely dissolve the RNA pellet.
8. **Swirl or shake the vial of Mag-Bind® oligo(dT) magnetic beads until the particles are in a homogeneous suspension.**
9. Transfer 50µl of **Mag-Bind® oligo(dT) magnetic beads** into a RNase-free microcentrifuge tube.
10. Add 150µl of 2 x Mag-Bind mRNA Binding Buffer, mix by pipetting. Incubate at 70°C for 3 minutes and then place at room temperature for 10 minutes.
11. Collect the magnetic beads by place the tube on a magnetic separation device (MSTD-02). The liquid should be cleared after the magnetic beads are completely pelleted.
12. Aspirate the supernatant by pipetting. Remove the tube from magnetic stand.
13. Wash the magnetic beads again by adding 200µl mRNA Wash Buffer. Resuspend the magnetic beads by vortexing for 20 seconds.
14. Collect the magnetic beads by place the tube on a magnetic separation device (MSTD-02). The liquid should be cleared after the magnetic beads are completely pelleted.
15. Aspirate the supernatant by pipetting. Dry the magnetic beads pellet by air for 5-10 minutes. Remove any liquid with a pipettor.
16. Remove the tube from magnetic stand and then add 100µl of mRNA elution Buffer to the particles. Incubate the tube at room temperature

with gentle agitation for 5 minutes to release mRNA from the magnetic particles.

17. Place the tube on a magnetic stand to collect magnetic particles.
18. Transfer the supernatant contains eluted mRNA into a RNase-free tube. The RNA can be store for -20°C for short term storage and -80°C for long term storage.
19. Precipitate mRNA by ethanol precipitation: Add 10µl of 5 M NaCl and 2 volume of cold absolute ethanol. Incubate 20 minutes at -20°C. Centrifuge at maximum speed for 10 minutes at room temperature. Wash once with 300 µl 70% ethanol and dissolve the purified mRNA with 10-20µl nuclease-free water.

E.Z.N.A.® Mag-Bind mRNA Protocol (Centrifugation Protocol)

Materials to be provided by user

- Microcentrifuge with adaptor for 1.5 ml tube
- Nuclease-free 1.5ml centrifuge tubes
- Absolute Ethanol, chilled at -20°C
- Nuclease-free 15 ml plastic conical centrifuge tubes
- Microcentrifuge
- Chloroform

1. **Prepare the total RNA and mix total RNA sample with Mag-Bind Oligo (dT) beads by following step 1-10 in the standard (magnate) protocol.**
2. Centrifuge at 8,000 x g for 1 minutes to collect magnetic particles.
3. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle pellet.
4. Wash the magnetic beads by adding 200 µl mRNA Wash Buffer.
5. Centrifuge at 8,000 x g for 1 minutes to collect magnetic particles.
6. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle pellet.
7. Wash the magnetic beads again by adding another 200 µl mRNA Wash Buffer.
8. Centrifuge at 8,000 x g for 1 minutes to collect magnetic particles.
9. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle pellet.

10. Centrifuge at 10,000 x g for 1 minutes to collect magnetic particles.
11. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle pellet.
12. Dry the magnetic beads pellet by air for 5-10 minutes. Remove any liquid with a pipettor.
13. Add 100µl of mRNA elution Buffer to the particles. Incubate the tube at room temperature with gentle agitation for 5 minutes to release mRNA from the magnetic particles.
14. Centrifuge at 10,000 x g for 2 minutes to collect magnetic beads.
15. Transfer the supernatant contains eluted mRNA into a RNase-free tube. The RNA can be store for -20°C for short term storage and -80°C for long term storage.
16. Optional: Precipitate mRNA by ethanol precipitation by adding 10µl of 5 M NaCl and 2.5 volume of cold absolute ethanol. Incubate 20 minutes at -20°C. Centrifuge at maximum speed for 10 minutes at room temperature. Wash once with 300 µl 70% ethanol and dissolve the purified mRNA with 10-20µl nuclease-free water.

Troubleshooting Guide

Problem	Cause	Suggestion
Degraded RNA	RNase contamination from handling	<ul style="list-style-type: none"> ● Follow protocol closely, and work quickly. ● Wear gloves throughout the procedure and when handling the solution and equipments used for RNA isolation.
	RNase contamination from total RNA sample	<ul style="list-style-type: none"> ● Ensure not to introduce RNase during the procedure. ● Check Total RNA sample for RNase contamination: incubate the total RNA sample at 65C for 5 minutes and them incubate at room temperature for 10 minutes. Analyze the 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	<ul style="list-style-type: none"> ● Ensure Total RNA sample is heated at 65C prior to addition of magnetic particles. ● If the rRNA level is too high for downstream application, purify the mRNA with second round purification with fresh magnetic particles.
OD260/OD280 ration is too low	Magnetic beads interference	<ul style="list-style-type: none"> ● Completely remove the magnetic particles by magnetic stand or centrifugation.