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Revised March 2008

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 throughly 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 2°C-8°C. Under these conditions, RNA has successfully been purified and used for RT-PCR after 12 months of storage. During shipping and storage, crystals may form in the Binding Buffer, simply warm to 37°C to dissolve. **Do not frozen the Mag-Bind® oligo(dT) magnetic beads solution.**

Binding Capacity

100 µl of the **Mag-Bind® oligo(dT) magnetic beads solution** can bind approximately 2-4 µg mRNA.

Kit Contents

Product No.	R6570-00	R6570-01	R6570-02
Purification	2	10	30
Oligo(dT) magnetic beads	210 µl	1.05 ml	3 x 1.5 ml
RNA-Solv® Reagent	5 ml	20 ml	60 ml
2 x mRNA Binding Buffer	5 ml	20 ml	60 ml
mRNA Wash Buffer	5 ml	20 ml	60 ml
mRNA Elution Buffer	1 ml	5 ml	5 ml
User Manual	1	1	1

Before Starting

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 37°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 # MSD-02)
- Nuclease-free 1.5 and 2 ml centrifuge tubes
- 80% Ethanol
- Isopropanol
- Microcentrifuge capable of 12,000 x g and 2-8°C
- Chloroform

This protocol is for isolating mRNA from up to **100 mg** tissue or **1 x 10^7 cells**. (Scale-up accordingly for larger samples) When scale up or down, simply increase or decrease the volume of all regents include the Mag-Bind® oligo(dT) magnetic beads.

A. Total RNA Isolation

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

   a) Tissue Samples

   Homogenize tissue samples in 1.5 mL of RNA-*Solv*® Reagent per 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 2 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-1015-00). 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 1.5 mL of the RNA-Solv Reagent per 1 x 10^7 of animal, plant or yeast cells, or per 1 x 10^8 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 1.5 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 (~1.5 mL per 10 cm^2). An insufficient amount of RNA-*Solv*® Reagent may result in contamination of the isolated RNA with DNA. Always use more RNA-*Solv*® Reagent if in the lysate is too viscous to aspirate with a pipette.

2. **Add 0.3 mL of chloroform per 1.5 mL of RNA-*Solv*® Reagent.** 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 12,000 x g for 10 minutes at 4°C.

4. **Precipitation of RNA.** Transfer no more than 80% of the aqueous phase to a fresh 2 ml tube, and discard the lower organic phase. Precipitate the RNA from the aqueous phase by **adding 750 µl of isopropanol per 1.5 mL of RNA-*Solv*® Reagent** used for the initial homogenization. Invert the sample 10-20 times. Incubate samples at room temperature 10 minutes and centrifuge at no more than 12,000 x g for 10 minutes at 2-8 °C.

5. **Discard the supernatant and wash the RNA pellet once with 1 ml 80% ethanol.** Mix the sample by vortexing and centrifuge at no more than 7,500 x g for 5 minutes at 2-8°C.

6. **Carefully aspirate and discard the ethanol. 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 200 µl RNase-Free water and vortex for 20 seconds.** Incubate at room temperature to completely dissolve the RNA pellet.

B. Oligo(dT) Magnetic beads Washing Procedure

8. Swirl or shake the vial of Oligo(dT) magnetic beads until the particles are in a homogeneous suspension.

9. **Transfer 100 µl of Oligo(dT) Magnetic beads into a new tube.** Place the tube on a magnetic separation device (MSD-02). The Oligo(dT) beads will migrate to the side of the tube nearest the magnet.

10. Remove the supernatant with a pipette while the tube remains on the magnet.

11. Remove the tube from the magnet **and add 400 µl of mRNA Wash Buffer to resuspend the beads.** Again place the tube on the magnet for 5 minutes.

12. Remove the supernatant while the tube remains on the magnet.

13. Remove the tube from the magnet and **200 µl of 2 x Binding Buffer to resuspend the beads.**

C. mRNA Purification

14. **Incubate the purified RNA at 65°C for 3 minutes** to disrupt secondary structures. Immediately place on ice for 3 minutes.

15. **Transfer 200 µl of the RNA from step 14** into the tube containing the beads. Mix throughly and then place on a rotating mixer for 10 minutes at room temperature.

16. **Place the tube on the magnet for 10 minutes.** The liquid should be cleared after the magnetic beads are completely magnetized.

17. Aspirate the supernatant by pipetting. Remove the tube from magnetic stand.

18. **Wash the magnetic beads by adding 300 µl mRNA Wash Buffer.** Resuspend the magnetic beads by pipetting carefully a couple of times.

19. Collect the magnetic beads by place the tube on a magnetic separation device (MSD-02). Carefully remove all the supernatant.

20. Repeat the washing step as described in step 18-19.

D. mRNA Elution

21. If the isolated mRNA does not need to be eluted off the beads, wash one more time using the same buffer that will be used in the downstream application, e.g. reverse transcription first strand synthesis buffer (without

the enzyme). Then resuspend the beads in an appropriate volume of the downstream buffer.

22. If the isolated mRNA elution is required, add the desired amount (10-30 µl) of mRNA Elution Buffer (10mM Tris, 1mM EDTA). Heat to 65°C for 2 minutes and place the tube immediately on the magnet. Quickly transfer the eluted mRNA to a new RNA-Free tube.

Troubleshooting Guide

Problem	Cause	Suggestion
Degraded RNA	RNase contamination from handling	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	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	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	Completely remove the magnetic particles by magnetic stand or centrifugation.