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.

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

Materials to be provided by user

- Nuclease-free 1.5ml centrifuge tubes
- Microcentrifuge

1. **Swirl or shake the vial of Mag-Bind® oligo(dT) magnetic beads until the particles are in a homogeneous suspension.**

2. **Transfer 50 µl of Oligo(dT) Magnetic beads into a new tube.** Centrifuge at 8,000 x g for 2 minutes at room temperature. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle pellet.

3. **Add 400 µl of mRNA Wash Buffer to resuspend the beads.** Centrifuge at 8,000 x g for 2 minutes at room temperature.

4. Carefully remove the supernatant with a pipettor and **add 100 µl of 2 x Binding Buffer to resuspend the beads.** Under cool ambient conditions, a precipitate may form in the 2 x mRNA Binding Buffer. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer 2 x mRNA Binding Buffer at room temperature.

5. **Prepare total RNA (100 µg) in 100 µl of mRNA Elution Buffer or 100 µl of Nuclease-Free water.**

   Note: if the concentration of total RNA is less than 1 µg/µl. The 100µg RNA will have volume large than 100 µl. In this case, increase the volume of Mag-Bind® mRNA Binding Buffer used in step 4 equal to the volume of total RNA sample.

6. **Transfer 100 µl of the RNA from step 5 into the tube containing the beads.** Incubate at 65°C for 2-3 minutes to disrupt secondary structures.

7. **Mix throughly and then place on a rotating mixer for 15 minutes at room temperature.**

8. Centrifuge at 8,000 x g for 2 minutes to collect magnetic particles. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle

Kit Contents

Product No.	R6520-00	R6520-01	R6520-02
Purification	2	10	30
Oligo(dT) Magnetic Beads	110 µl	550µl	1.6 ml
2 x Mag-Bind mRNA Binding Buffer	500 µl	5 ml	15 ml
Mag-Bind mRNA Wash Buffer	5 ml	20 ml	60 ml
mRNA Elution Buffer	500 µl	1.5 ml	5 ml
User Manual	1	1	1

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.

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 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 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 kit, except oligo(dT) magnetic beads should be stored at 22-25ºC. Oligo(dT) magnetic beads should be stored at 2-8ºC. Under these conditions, RNA has successfully been purified and used for RT-PCR after 12 months of storage. **Do not frozen the Mag-Bind oligo(dT) magnetic beads solution .**Under cool ambient conditions, a precipitate may form in the 2 x mRNA Binding Buffer. In case of such an event, heat the bottle at 37˚C to dissolve. Store Buffer 2 x mRNA Binding Buffer at room temperature.

Binding Capacity

50ul of the **Mag-Bind® oligo(dT) magnetic beads solution** can bind approximately 2µg mRNA.

pellet.

9. **Wash the magnetic beads again by adding 400 µl mRNA Wash Buffer.** Resuspend the magnetic beads by vortexing or pipetting.

10. Centrifuge at 8,000 x g for 2 minutes to collect magnetic particles. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle pellet.

11. **Wash the magnetic beads again by adding another 400 µl mRNA Wash Buffer.** Resuspend the magnetic beads by vortexing or pipetting.

12. Centrifuge at 8,000 x g for 2 minutes to collect magnetic particles. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle pellet.

13. **Dry the magnetic beads pellet by air for 5-10 minutes.**

14. **Add 15-50 µl of mRNA Elution Buffer to the particles.** Resuspend the magnetic beads by vortexing or pipetting. Incubate the tube at room temperature with gentle agitation for 5 minutes to release mRNA from the magnetic particles.

15. Centrifuge at 8,000 x g for 2 minutes to collect magnetic beads.

16. **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.

E.Z.N.A.™ 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

1. **Swirl or shake the vial of Mag-Bind® oligo(dT) magnetic beads until the particles are in a homogeneous suspension**.

2. **Transfer 50 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. Remove the supernatant with a pipette while the tube remains on the magnet.

3. **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. Remove the supernatant while the tube remains on the magnet.

4. **Remove the tube from the magnet and add 100 µl of 2 x mRNA Binding Buffer to resuspend the beads.** Under cool ambient conditions, a precipitate may form in the 2 x mRNA Binding Buffer. In case of such an event, heat the bottle at 37°C to dissolve.

5. **Prepare total RNA (100 µg) in 100 µl of mRNA Elution Buffer or 100 µl of Nuclease-Free water.**
   Note: if the concentration of total RNA is less than 1 µg/µl. The 100µg RNA will have volume large than 100 µl. In this case, increase the volume of Mag-Bind® mRNA Binding Buffer used in step 4 equal to the volume of total RNA sample.

6. **Transfer 100 µl of the RNA from step 5 into the tube containing the beads.** Incubate at 65°C for 2-3 minutes to disrupt secondary structures.

7. Mix throughly and then place on a rotating mixer for 15 minutes at room temperature.

8. **Collect the magnetic beads by place the tube on a magnetic separation device.** The liquid should be cleared after the magnetic beads are completely pelleted. Aspirate the supernatant by pipetting.

9. **Remove the tube from magnetic stand and wash the magnetic beads by adding 400 µl mRNA Wash Buffer.** Resuspend the magnetic beads by vortexing or pipetting.

10. **Collect the magnetic beads by place the tube on a magnetic separation device.** The liquid should be cleared after the magnetic beads are completely pelleted. Aspirate the supernatant by pipetting.

11. **Remove the tube from magnetic stand and wash the magnetic beads again by adding another 400 µl mRNA Wash Buffer.** Resuspend the magnetic beads by vortexing or pipetting.

12. **Collect the magnetic beads by place the tube on a magnetic separation device.** The liquid should be cleared after the magnetic beads are completely pelleted. Aspirate the supernatant by pipetting.

13. **Dry the magnetic beads pellet by air for 5-10 minutes.** Remove any liquid with a pipettor.

14. **Remove the tube from magnetic stand and then add 15-50 µl of mRNA Elution Buffer to the particles.** Resuspend the magnetic beads by vortexing or pipetting. Incubate the tube at room temperature with gentle agitation for 5 minutes to release mRNA from the magnetic particles.

15. Place the tube on a magnetic stand to collect magnetic particles.

16. **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.