

Contents

Introduction.....	2
New In This Edition.....	2
Storage and Stability.....	2
Binding Capacity.....	2
Kit Contents.....	3
Before Starting.....	3
Working with RNA.....	3
E.Z.N.A.® Mag-Bind mRNA Protocol (Magnetic stand protocol).....	4
E.Z.N.A.® Mag-Bind mRNA Protocol (Centrifugation protocol).....	6
Troubleshooting Guide.....	8

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 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 E.Z.N.A.® Mag-Bind mRNA Enrichment 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.	R6520-00	R6520-01	R6520-02
Purification	2	10	30
Oligo(dT) Magnetic Beads	110 µl	550µl	1.5 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

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.

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
- Absolute Ethanol, chilled at -20°C
- Microcentrifuge

1. Prepare the total RNA (100:g) in 100µl of mRNA Elution Buffer or 100µl of Nuclease-Free water.

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

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

3. Heat the total RNA sample to 65°C for 4 minutes.
4. Transfer 50µl of **Mag-Bind® oligo(dT) magnetic beads** into the total RNA sample.
5. 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.
6. 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.
7. Aspirate the supernatant by pipetting. Remove the tube from magnetic stand.
8. Wash the magnetic beads again by adding 200µl mRNA Wash Buffer. Resuspend the magnetic beads by vortexing for 20 seconds.
9. 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.
10. Aspirate the supernatant by pipetting. Dry the magnetic beads pellet by air for 5-10 minutes. Remove any liquid with a pipettor.
11. 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.
12. Place the tube on a magnetic stand to collect magnetic particles.
13. 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.
14. 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
 - Microcentrifuge
1. Prepare the total RNA (100:g) in 100µl of mRNA Elution Buffer or 100µl of Nuclease-Free water.

Note: if the concentration of total RNA is less than 1:g/:l. The 100:g RNA will have volume large than 100:l. In this case, increase the volume of Mag-Bind mRNA Binding Buffer used in step 4 equal to the initial volume of total RNA sample
 2. **Swirl or shake the vial of Mag-Bind® oligo(dT) magnetic beads until the particles are in a homogeneous suspension.**
 3. Transfer 50µl of **Mag-Bind® oligo(dT) magnetic beads** into the total RNA sample.
 4. 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
 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 by adding 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. Wash the magnetic beads again by adding another 200 µl mRNA Wash Buffer.
 11. Centrifuge at 8,000 x g for 1 minutes to collect magnetic particles.
 12. Carefully remove the supernatant with a pipettor. Avoid disturb

the magnetic particle pellet.

13. Centrifuge at 10,000 x g for 1 minutes to collect magnetic particles.
14. Carefully remove the supernatant with a pipettor. Avoid disturb the magnetic particle pellet.
15. Dry the magnetic beads pellet by air for 5-10 minutes. Remove any liquid with a pipettor.
16. 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. Centrifuge at 10,000 x g for 2 minutes to collect magnetic beads.
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. 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 then 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.