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Revised July 2005

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 sample of up to 1mg. 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 accommodates both small (50µg) and large (up to 1mg) total RNA samples, 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 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.	R6520-01	R6520-02
Purification	10	30
Mag-Bind oligo(dT) magnetic beads solution Beads	500µl	1.5 ml
2 x Mag-Bind mRNA Binding Buffer	5 ml	15 ml
1 x Mag-Bind mRNA Binding	3 ml	10 ml
Mag-Bind mRNA Wash Buffer	3 ml	10 ml
mRNA Elution Buffer	1.5 ml	5 ml
User Manual	1	1

Before Starting

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 (OBI # MSTD-01)
- Nuclease-free 1.5ml centrifuge tubes

This protocol is for isolating mRNA from 100µg total RNA. Reactions can be either scale down to 50µg total RNA or up to 1 mg total RNA in a single microcentrifuge tube. When scale up or down, simply increase or decrease the volume of all reagents include the Mag-Bind oligo(dT) magnetic beads.

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

Note: if the concentration of total RNA is less than 1µg/µl. The 100µg RNA will have volume large than 100µl. 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. **Heat the total RNA sample to 65°C for 4 minutes.**
3. **Swirl or shake the vial of Mag-Bind® oligo(dT) magnetic beads until the particles are in a homogeneous suspension.**
4. Transfer 50µl of **Mag-Bind® oligo(dT) magnetic beads** into a RNase-free microcentrifuge tube.
5. Add 100µl 2 x Mag-Bind mRNA Binding Buffer, mix by pipetting.
6. Collect the magnetic beads using a magnetic separation stand, according to the manufacturer's instruction.
7. Aspirate the supernatant by pipetting. Remove the tube from magnetic stand.
8. Wash the magnetic beads again by repeating step 5-7.
9. Resuspend the magnetic beads with 100µl **2 x Mag-Bind mRNA Binding Buffer.**
10. Add 100µl of total RNA solution to the tube contains suspended magnetic beads solution. Mix by pipetting.
11. Incubate the mixture at room temperature for 5 minutes with gently agitation to allow hybridization of poly(A) RNA to the

- beads.
12. Collect the magnetic beads/mRNA complex with magnetic stand.
 13. Remove the supernatant with a pipettor.
 14. Wash the mRNA-bound magnetic beads 2 times using 100µl **1 x mRNA Binding Buffer** for each wash. Gently resuspend the particles during each wash and then re-collect the particles using magnetic separation stand.
 15. Wash the mRNA-bound magnetic beads 2 times using **100µl mRNA Wash Buffer** for each wash. Gently resuspend the particles during each wash and then re-collect the particles using magnetic separation stand
- Note: Make sure to remove all supernatant when completing the final wash.**
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.

E.Z.N.A.[™] Mag-Bind mRNA Protocol (Centrifugation Protocol)

Materials to be provided by user:

- Microcentrifuge capable of at least 10,000 x g
- Nuclease-free 1.5ml centrifuge tubes

This protocol is for isolating mRNA from 100µg total RNA. Reactions can be either scale down to 50µg total RNA or up to 1 mg total RNA in a single microcentrifuge tube. When scale up or down, simply increase or decrease the volume of all reagents include the Mag-Bind oligo(dT) magnetic beads.

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

Note: if the concentration of total RNA is less than 1µg/µl. The 100µg RNA will have volume large than 100µl. 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. **Heat the total RNA sample to 65°C for 4 minutes.**
3. **Swirl or shake the vial of Mag-Bind® oligo(dT) magnetic beads until the particles are in a homogeneous suspension.**
4. Transfer 50µl of **Mag-Bind® oligo(dT) magnetic beads** into a RNase-free microcentrifuge tube.
5. Add 100µl 2 x Mag-Bind mRNA Binding Buffer, mix by pipetting.
6. Centrifuge at 10,000 x g for 1 minutes to collect magnetic particles.
7. Carefully Aspirate the supernatant by pipetting. Avoid disturb the magnetic particle pellet.
8. Wash the magnetic beads again by repeating step 5-7.
9. Resuspend the magnetic beads with 100µl **2 x Mag-Bind mRNA Binding Buffer.**
10. Add 100µl of total RNA solution to the tube contains suspended magnetic beads solution. Mix by pipetting.
11. Incubate the mixture at room temperature for 5 minutes with gently agitation to allow hybridization of poly(A) RNA to the beads.

12. Collect the magnetic beads/mRNA complex by centrifuge at 10,000 x g for 2 minutes.
13. Remove the supernatant with a pipettor.
14. Wash the mRNA-bound magnetic beads two times using 100µl of **1 x mRNA Binding Buffer** for each wash. Gently resuspend the particles during each wash and then re-collect the particles by centrifugation.
15. Wash the mRNA-bound magnetic beads two times using 100µl of **mRNA Wash Buffer** for each wash. Gently resuspend the particles during each wash and then re-collect the particles by centrifugation.

Note: Make sure to remove all supernatant when completing the final wash.
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. Centrifuge at 10,000 x g for 2 minutes 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

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.