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Introduction

E.Z.N.A.® Mag-Bind RNA Clean-up Kit provides a rapid and easy method for the purification and concentrate RNA from enzymatic reactions or for desalting the RNA samples. Up to 10 µg or down to picogram of RNA can be recovered with specially designed Mag-Bind Particles. RNA purified using E.Z.N.A.® Mag-Bind RNA Clean-up Kit is ready for all downstream applications such as RT-PCR*, Northern blotting, poly A^+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

Storage and stability

Mag-Bind® RNA Clear Up Kit are stable for at least 9 months from the date of purchase when stored at 2-8ºC.

Kit Contents

Product Number	M6248-01	M6248-02
Purification Times	50 Preps	200 Preps
Mag-Bind RNA Particles	0.55 ml	2.1 ml
Binding Solution	15 ml	50 ml
DEPC Treated H_2O	5 ml	20 ml
Instruction Manual	1	1

Before Starting

Please take a few minutes to read this booklet thoroughly and 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. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- During the procedure work carefully but quickly.
- Maxium starting sample should be limited to 10µg or 100µl due to the capacity of Mag-Bind Particles.

Mag-Bind RNA Clean-up Single Tube Protocol

This protocol is designed to recovery RNA from enzymatic reactions such as DNase I digestion, In vitro transcription, etc.

1. **Measure the volume of sample and adjust the sample volume to 100µl with DEPC Treated Water and proceed to step 2.**
2. **Add 200 µL Binding Solution and 10 µL MagBind Particles.** Mix well and incubate at room temperature for 10 min. Invert to mix occasionally during the incubation.
3. **Place the sample on a magnetic separation device for 10 minutes or until the solution clears.** If using Omega Bio-Tek's MSD-02 magnet, the magnetic beads will form a pellet adjacent to the magnet.
4. **Aspirate and discard the cleared supernatant.**
5. **With the tube on the magnet, add 500µl 70% ethanol, shaking vigorously to resuspend the beads.** Sit for 5 minutes or until the magnetic beads is fully resettled.
6. **Aspirate and discard the supernatant.**
7. **With the tube on the magnet, add another 500µl 70% ethanol, shaking vigorously to resuspend the beads.** Sit for 5 minutes or until the magnetic beads is fully resettled.
8. **Aspirate and discard the supernatant.** Sit for 2 minutes and remove the supernatant completely.
9. **Air dry the magnetic particles for 10 minutes.**
10. **Add 20-50 µl DEPC Treated Water.** Mix throughly by vortexing. Incubate at room temperature for 5 minutes.
11. **Place the tube onto a magnetic separation device and wait 10 minutes or until the magnetic beads are cleared from solution.**

12. **Transfer the cleared supernatant** contains purified sequencing product into a new tube.

13. Store the purified RNA at -80oC.

Mag-Bind RNA Clean-up 96 Protocol

This protocol is designed to recovery RNA from enzymatic reactions such as DNase I digestion, In vitro transcription, etc.

1. **Transfer RNA into 96 well 0.5ml Plate and adjust the sample volume to 50µl with DEPC-Water and proceed to step 2.**

2. **Add 100 µL Binding Solution and 10 µL MagBind Particles.** shaking to mix and incubate at room temperature for 10 min. Shaking to mix occasionally during the incubation.

3. **Place the sample plate on a magnetic separation device for 10 minutes or until the solution clears.** If using Omega Bio-Tek's MSD-01 magnet, the magnetic beads will form a pellet at corner of each well adjacent to the magnet.

4. Aspirate and discard the cleared supernatant.

5. **Remove the plate from the magnet, add 200µl 70% ethanol to each well, shaking to mix for 2 minutes.**

6. Place the sample plate on a magnetic separation device for 10 minutes or until the solution clears.

7. Aspirate and discard the cleared supernatant.

8. **Remove the plate from the magnet, add 200µl 70% ethanol to each well, shaking to mix for 2 minutes.**

9. Place the sample plate on a magnetic separation device for 10 minutes or until the solution clears.

10. **Aspirate and discard the supernatant.**
    Note: It is critical to completely remove all liquid from each well since it contains ethanol and other contaminants.

11. **Air dry the magnetic particles for 10 minutes.**
12. **Add 20-50 µl DEPC Treated Water.** Shaking to mix for 3 mintes.

13. Incubate at room temperature for 5 minutes.

14. **Place the plate onto a magnetic separation device and wait 7-10 minutes or until the magnetic beads are cleared from solution.**

15. **Transfer the cleared supernatant** contains purified sequencing product into a new microplate.

16. Store the purified RNA at -80oC.

Bioanalyser by comparison the ration of 28S and 18S RNA.

Troubleshooting Guide

Problem	Cause	Suggestion
Little or no RNA eluted	**RNA remains on MagBind Particles**	**Repeat elution.** **Pre-heat DEPC-water to 65°C prior to elution.** **Incubate column for 10 min with water prior to centrifugation.**
Degraded RNA	RNase contamination	Ensure not to introduce RNase during the procedure. Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	Repeat wash with 70% ethanol
DNA contamination		Digest with RNase-free DNase and inactivate at 75°C for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	DEPC-treated water is acidic and can dramatically lower Abs260 values. Use TE buffer to dilute RNA prior to spectrophotometric analysis.