Contents

Introduction

The E.Z.N.A.™ Mag-Bind® Total Nucleic Acid Kit is designed for rapid and reliable isolation of Total Nucleic Acid from Whole Blood, Serum, Plasma, Saliva and other Body Fluids. The Mag-Bind® Magnetic Beads technology provide a high quality RNA or DNA, which is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. This system can be easily adapted with automated system or centrifugation system. The procedure can be scaled up or down, allowing purification from various amounts of starting materials.

Principle

If using the E.Z.N.A.™ Mag-Bind® Total Nucleic Acid Kit for the first time, please read this booklet to become familiar with the procedure and its various modification. Samples are lysed in a specially formulated buffer containing detergent. Nucleic acid was bound to the surface of Mag-Bind® magnetic particles under proper condition. Proteins and cellular debris are efficiently washed with few wash steps. Pure RNA and DNA is then eluted in nuclease-free water or low ionic strength buffer. Purified RNA or DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E.Z.N.A.™ Mag-Bind® Total Nucleic acid Kit are stable for at least 24 months from the date of purchase when stored at 8oC-25oC. During shipment, or storage in cool ambient conditions, precipitates may form in TNA Lysis Buffer. It is possible to dissolve such deposits by warming the solution at 37oC, though we have found that they do not interfere with overall performance.

Kit Contents

Product Number	M6258-00	M6258-01	M6258-02
Purifications	5 Preps	50 Preps	200 Preps
Mag-Bind® Magnetic Beads T	120 µl	1.2 ml	4 x 1.2 ml
TNA Lysis Buffer	5 ml	20 ml	60 ml
Buffer VHB	4 ml	17 ml	66 ml
Carrier RNA	-	1 mg	2 x 1 mg
OB Proteinase	120 µl	1.2 ml	4 x 1.2 ml
SPR Wash Buffer	3 ml	2 x 30 ml	3 x 30 ml
Nuclease free Water	1 ml	10 ml	20 ml
User Manual	1	1	1

Before Starting

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Wear gloves/ protective goggles and take great care when working with chemicals.

IMPORTANT

1 **Add Nuclease free water to the tube containing lyophilized carrier RNA to obtain a solution of 1 µg/µl.** Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

2 **SPR Wash Buffer must be diluted with absolute ethanol before use.**

M6258-00	Add 7 ml 96-100% ethanol
M6258-01	Add 70 ml 96-100% ethanol per bottle
M6258-02	Add 70 ml 96-100% ethanol per bottle

3 **VHB Buffer must be diluted with absolute ethanol before use.**

M6258-00	Add 6 ml 96-100% ethanol
M6258-01	Add 23 ml 96-100% ethanol per bottle
M6258-02	Add 84 ml 96-100% ethanol per bottle

E.Z.N.A.® Mag-Bind® Total Nucleic acid Automatic Protocol

This method allows total Nucleic acid isolation from up to 200 µl serum, plasma, whole blood, saliva or other body fluids. Yields vary depending on source.

- **Materials to be provided by user:**
  - ✓ Nuclease-free 1.5 ml centrifuge tube or 96 well plate
  - ✓ Isopropanol
  - ✓ Magnetic separation strand

- **(Optional) Addition of carrier RNA to Lysis Buffer: Carrier RNA and TNA Lysis Buffer can be premixed. Prepare TNA Lysis Buffer/Carrier RNA always fresh prepared before used. Do not store. Thaw one vial of poly(A) carrier RNA. Transfer 4 µl Carrier RNA into 1 ml TNA Lysis Buffer.** when processing cell free sample such as serum, cell free saliva, cell free body fulids, Carrier RNA should be added. When processing cell contained sample, such as whole blood, saliva, Carrier RNA should not be added.

1. **Transfer 20 µl Proteinase K into a 1.5 ml tubes or each well of 96 deep well plate.**

2. **Add 200 µl of plasma or serum into the tube or the plate.** If using pre-frozen samples, thaw at room temperature (15-20°C) and mix well by shaking or pipetting up and down before proceeding step 3. **If the sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution to bring the volume of protease and sample up to a total of 220 µl.**

3. **Add 200 µl of TNA Lysis Buffer/Carrier RNA to the sample.** Mix by shaking 30 seconds. Incubate at room temperature for 10 min to completely lyse samples.

4. **Add 20 µl Mag-Bind® Beads T and 440 µl of isopropanol.** Mix by shaking for 5 minutes.

5. **Place the tube on a magnetic separation device suitable for 1.5 ml tube or 96-well plate to magnetize the Mag-Bind® particles for 10-15 min.**

6. Aspirate the cleared solution (supernatant) from the tube or plate and discard.

   This step must be performed while the plate is situated on the magnet. To avoid disturbing the beads, place the pipette tip at the bottom of the tube when aspirating. Remove as much supernatant as possible.

7. Remove the tube or plate containing the Mag-Bind®particles from the magnetic separation device. **Add 500 µl Buffer VHB into the tube or each well of plate.**

   Note: Buffer VHB must be diluted with absolute ethanol before use. Refer to label on bottle for instruction.

8. **Resuspend Mag-Bind® particles pellet by shaking for 2 minutes**.

9. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles for 5 min.

10. Aspirate the cleared solution (Supernatant) from the tube or plate and discard.

    This step must be performed while the plate is situated on the magnet. To avoid disturbing the beads, place the pipette tip at the bottom of the tube when aspirating. Remove as much supernatant as possible.

11. Remove the tube or plate containing the Mag-Bind®particles from the magnetic separation device. **Add 500 µl of SPR Wash Buffer into the tube or each well of plate.**

12. **Resuspend Mag-Bind® particles pellet by shaking for 2 minutes**.

13. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles for 5 min.

14. Aspirate the cleared solution (Supernatant) from the tube or plate and discard.

15. **Repeat Step 11-14 for a total of two 500 µl of SPR Wash Buffer washes.**

16. Leave the tube or plate air dry for 10 minutes on the magnetic separation device.

17. **Remove the tube from magnetic separation device. Add 20-50 µl Nuclease**

free Water.

18. **Resuspend Mag-Bind® particles by** shaking for 2 minutes.

19. Incubate at room temperature for 10 minutes.

20. Place the tube onto a magnetic separation device to magnetize the Mag-Bead® particles.

21. Transfer the cleared supernatant containing purified DNA to a new 1.5 ml tube or plate.

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low RNA yields	Incomplete resuspension of magnetic particle	Resuspend the magnetic particles by vortexing before use.
	RNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	SPR Buffer were not prepared correctly.	Prepare the SPR by adding ethanol according to instruction
	Lose of magnetic beads during operation	carefully not remove the magnetic beads during the operation
	Inefficient cell lysis due to decrease of activity of proteinase K	Add more proteinase K solution.
No DNA eluted.	SPR Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare SPR Wash Buffer Concentrate as instructed on the label.
Problem with downstream application	Insufficient RNA was used	1. RNA in the sample already degraded, do not frozen and thaw the sample more than once or store at room temperature for too long 2. Quantify the purified RNA accurately and use sufficient DNA.
Carryover of the magnetic beads in the elution	Carryover the magnetic beads in the eluted RNA will not effect downstream applications.	To remove the carryover magnetic beads from eluted RNA, simply magnetize the magnetic beads and carefully transfer to a new tubes or plate.