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Introduction

The E.Z.N.A.® Soil RNA Kit allows rapid and reliable isolation of high- quality total RNA from various soil samples. The system combines the reversible nucleic acid-binding properties of HiBind™ matrix with the OBI proprietary soil nucleic acid purification technology to eliminate inhibitor compounds such as humic acid and fulvic acid from soil samples. Purified RNA is suitable for most downstream applications such as RT-PCR.

Overview

If using the E.Z.N.A.® Soil RNA Kit for the first time, please read this booklet to become familiar with the procedure. Soil sample is homogenized and then treated in a specially formulated buffer containing detergent and glass beads. Humic acid, proteins, polysaccharides, and other contaminants are effectively removed with precipitation and phenol extraction steps. Binding conditions are then adjusted and the sample is applied to an HiBind™ RNA spin-column. Two rapid wash steps remove trace contaminants and pure RNA is eluted in DEPC treated water. Purified RNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E.Z.N.A.® Soil RNA Kit should be stored at room temperature. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by incubation the solution at 65ºC. All kit components are guaranteed for 12 months from date of purchase.

Kit Contents

Product Number	R6825-00	R6825-01	R6825-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® RNA mini Columns	5	50	200
2 mL Collection Tubes	10	100	40
Glass Beads I (0.1-0.2mm)	5 g	60 g	250 g
Glass Beads II (0.4-0.6mm)	5 g	60 g	250 g
Buffer SRX	20 mL	120 mL	250 mL
Buffer DS	2 mL	20 mL	25ml
RNA-Solv Reagent	7 mL	60 mL	220 mL
RWC Wash Buffer	5 mL	50 mL	200 mL
RNA Wash Buffer II	2 mL	12 mL	4 X 12 mL
DEPC Water	1.5 mL	20 mL	80 mL
Instruction Booklet	1	1	1

Before Starting

- **Prepare the water-saturated phenol solution:** Place the solid phenol into the water bath preset at 65°C until phenol is completely dissolved. Add equal volume of molecular biology grade water and mix throughly by shaking. Store the solution at room temperature for 4 hours to overnight until the water phase (upper phase) and phenol phase (lower phase) are clearly separated. Remove the water phase with transfer pipette.

- **Dilute RNA Wash Buffer II with absolute ethanol as follows and store at room temperature.**

R6825-00	Add 8 mL (96%-100%) ethanol.
R6825-01	Add 48 mL (96%-100%) ethanol to each bottle.
R6825-02	Add 48 mL (96%-100%) ethanol to each bottle.

E.Z.N.A. ® Soil RNA Kit Protocol

Materials to be provided by user

- Microcentrifuge capable of at least 14,000 x g
- Table top centrifuge capable of at least 3,000 x g
- Rotor for 15ml tube
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Nuclease-free 15 ml centrifuge tube
- Absolute (96%-100%) ethanol and 70% ethanol
- Water saturated phenol
- Chloroform

1. **Weigh 1 g glass beads I and 1 g glass beads II in a 15 mL centrifuge tube, add 2.5g soil sample.**

2. **Add 2ml Buffer SRX to the tube** and vortex for 1 min to mix the sample. Note: Shaking the Bottle of Buffer SRX to resuspend the solution before used.

3. **Add 200 μl of Buffer DS to the tube** and vortex for 30 seconds mix the sample.

4. **Add 1 mL of water-saturated phenol and followed by 1 ml Chloroform** and vortex at maxi speed for 10 minutes. For the best result, beads mixer such as FastPrep-24 should be used.

5. Centrifuge at 4,000 x g for 10 minutes at 4oC.

6. Carefully transfer the upper aqueous phase to a new 15 ml tube. Do not transfer the interface or phenol.

7. **Add equal volume of isopropanol** and mix throughly by invert the tube 10 times. Incubate at -20°C for 10 minutes.

8. Centrifuge at 4,000 x g for 20 minutes at at 4oC.

9. Remove the supernatant and invert the tube on a absorbent paper for 5 minutes to drain the liquid. It is not necessary to dry the pellet.

10. **Add 100µl DEPC water to the tube** and incubate at room temperature for 10 minutes. Mix the sample 2 times by vortexing during the incubation.

    Note: The pellet may be difficult to resuspend for some soil samples. Vortex the sample at maximum strength and incubate at 50°C for 10 minutes.

11. **Add 0.9ml RNA-Solv Reagent and 0.2 ml Chloroform** and mix throughly by vortexing for 15 seconds.

12. Centrifuge at 10,000 x g for 10 minutes at 4oC.

13. Carefully transfer the upper aqueous phase (about 0.5 ml) to a new 2 ml microtube.

14. **Add equal volume of 70% ethanol** and mix throughly by vortexing for 15 seconds.

15. Apply 750µl sample from step 14 into a HiBind RNA column inserted in a 2 ml collection tube.

16. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through and reuse the collection tube.

17. Place the column into same collection tube from previous step and load remaining sample from step 14.

18. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through and the collection tube.

19. Place the column into a new 2 ml collection tube. Add 500 µl of RWC Wash Buffer into the column. Centrifuge at 10,000 x g for 30 seconds. Discard the flow through and re-use the collection tube.

20. Place the column into the same collection tube from previous step. Add 750µl of RNA Wash Buffer II into the column. Centrifuge at 10,000 x g for 30 seconds. Discard the flow through and re-use the collection tube.

21. Place the column into same collection tube from previous step and centrifuge the empty column for 2 minute at maximum speed (>10,000 x g).

22. Place the HiBind RNA column into a new 1.5 ml microtube.

23. **Apply 30-50µl DEPC treated water at the center of the membrane of the column.** Incubate at room temperature for 1 minute.

24. Centrifuge at the maximum speed (>14,000 x g) for 1 minute to elute the RNA.

Troubleshooting Guide

Problem	Cause	Suggestions
A260/230 ratio is low	inefficient elimination of inhibitory compounds	Repeat the RNA isolation with a new sample, be sure to use less starting amount of material.
	No ethanol added to the lysate before loading to the column	Repeat the RNA isolation with a new sample.
	RNA wash Buffer II prepared with lower percentage ethanol	prepare RNA Wash Buffer with 96-100% ethanol
Low RNA yield or no RNA eluted	Sample stored incorrectly	Use fresh sample
	Poor lysis and homogenization of sample.	Repeat the RNA isolation with a new sample, be sure to vortex enough after the addition of SRC.
	Incorrect Ethanol was added before loading to the column	Check the ethanol and make sure to use 200 proof ethanol.
	RNA washed off.	Dilute RNA Wash Buffer II by adding appropriate volume of absolute ethanol prior to use (page 3).
	Ethanol residue in the elute	Be sure to completely dry the column before elution
Little or no supernatant after initial centrifuge step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary
sample can not pass through the column	Clogging column	Check the centrifugal force and increase the time of centrifugation
Pellet is difficult to resuspend on step 15.	Too much starting material	Double the volume of DEPC water and incubate the sample at 50C for 20-30 minutes.

Please feel free to contact our technical specialists at:

US customers: 800-832-8896 or 770-931-8400

All other customers: (770) 931-8400

Or direct your questions via E-mail to info@omegabiotek.com.

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