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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 65°C. 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 Columns	5	50	200
2 mL Collection Tubes	15	150	600
Glass Beads	5 g	50 g	200 g
Buffer SRA	20 mL	160 mL	650 mL
Buffer SRB	2 mL	16 mL	65 mL
Buffer SRC	7 mL	55 mL	210 mL
Buffer SRD	25 mL	250 mL	1000 mL
RNA-Solv Reagent	5 mL	50 mL	200 mL
RNA Wash Buffer I	5 mL	50 mL	500 mL
RNA Wash Buffer II	1 mL	10 mL	40 mL
DEPC Water	1.5 mL	20 mL	80 mL
Instruction Booklet	1	1	1

Before Starting

Please read the entire booklet to become familiar with the E.Z.N.A.® Soil RNA Kit protocol.

- **Prepare the water-saturated phenol solution:** Place the solid phenol into the water bath preset at 75°C until phenol is completely dissolved. Add equal volume of molecular biology grade water and mix thoroughly 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 4 mL (96%-100%) ethanol.
D6825-01	Add 40 mL (96%-100%) ethanol to each bottle.
D6825-02	Add 160 mL (96%-100%) ethanol to each bottle.

- Add Water-Saturated-Phenol to the bottle of RNA-Solv Reagent

R6825-00	Add 7.5 ml Water-Saturated-Phenol
R6825-01	Add 75 ml Water-Saturated-Phenol
R6825-02	Add 300 ml Water-Saturated-Phenol

- Preheat Heating block or water bath at 65°C

E.Z.N.A.® Soil RNA Kit Protocol

Materials to be provided by user

- Microcentrifuge capable of at least 14,000 xg
 - Table top centrifuge capable of at least 3,000 xg
 - Rotor for 15ml tube
 - Nuclease-free 1.5 mL or 2 mL microfuge tubes
 - Nuclease-free 15 ml centrifuge tube
 - Water bath equilibrated to 65°C
 - Absolute (96%-100%) ethanol
 - Water saturated phenol
 - Chloroform:Isoamyl alcohol (24:1)
1. Weigh 1 g of glass beads in a 15 mL centrifuge tube, add 2g soil sample.
 2. Add 3 ml Buffer SRA to the tube and vortex vigorously for 1 minutes to mix the sample.
 3. Add 300µl of Buffer SRB to the tube and mix thoroughly by vortexing for 30 seconds.

4. Add 1 mL of Buffer SRC and mix thoroughly by vortexing for 3 minutes.
5. Add 2.1 mL of water-saturated phenol and followed by 2.1 ml Chloroform : Isoamyl alcohol (24:1) and mix vigorously for 2 minutes.
6. Incubate the tube at 65°C for 10 minutes. Mix the sample 1-2 times during the incubation.
7. Centrifuge at 3000 x g for 10 minutes at room temperature.
8. Carefully transfer the upper aqueous phase to a new 15 ml tube. Do not transfer the interface or phenol.
9. Add 0.5 volume of Buffer SRD and mix thoroughly by vortexing. incubate at 4°C for 10 minutes.
10. Centrifuge at 3000 x g for 20 minutes at room temperature.

Note: If high speed centrifuge is available, centrifuge at 13,000 x g for 10 minutes can reduce the color of sample in the downstream steps of this protocol.

11. Carefully transfer the supernatant to a new 15 ml tube.
12. Add equal volume of isopropanol and mix thoroughly by invert the tube 10 times. Incubate at -20°C for 10 minutes.
13. Centrifuge at 3000 x g for 30 minutes at room temperature.

Note: If high speed centrifuge is available, centrifuge at 13,000 x g for 20 minutes can improve the RNA yield. The pellet may show dark color at this stage, it will not effect the RNA quality.

14. 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.
15. Add 200µl 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 50C for 10-20 minutes.

16. Transfer the sample into a new 15 ml tube. Add 1.8ml RNA-Solv Reagent. (See page 3 for instruction to prepare RNA-Solv Reagent).
17. Add 0.4 ml Chloroform and mix thoroughly by vortexing for 10 seconds.
18. Centrifuge at 3000 x g for 10 minutes at room temperature.
19. Carefully transfer the upper aqueous phase (about 1 ml) to a new 2 ml microtube.
20. Add 0.5 volume of absolute ethanol (96-100%) and mix thoroughly by vortexing for 10 seconds.
21. Apply 750µl sample from step 20 into a HiBind RNA column inserted in a 2 ml collection tube.
22. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through and reuse the collection tube.
23. Place the column into same collection tube from previous step and load remaining sample from step 20.
24. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through and the collection tube.
25. Place the column into a new 2 ml collection tube. Add 500µl of RNA Wash Buffer I into the column. Centrifuge at 10,000 x g for 30 seconds. Discard the flow through and re-use the collection tube.
26. 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.
27. Place the column into same collection tube from previous step and centrifuge the empty column for 2 minute at maximum speed (>14,000 x g).
28. Place the HiBind RNA column into a new 1.5 ml microtube.
29. Apply 50-100µl DEPC treated water at the center of the membrane of the column. Incubate at room temperature for 1 minute.
30. 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:

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

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