Contents

Introduction. 2

Overview. 2

Kit Contents. 3

Storage and Stability. 3

Before Starting. 4

Materials to Be Provided by User. 5

E.Z.N.A.® Soil RNA Mini Kit Protocol. 5

Trouble Shooting Guide. 7

Introduction

The E.Z.N.A.® Soil RNA Mini 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 Mini 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 a HiBind® RNA Mini Column. Three rapid wash steps remove trace contaminants and pure RNA isolated in DEPC Water. Purified RNA can be directly used in downstream applications without the need for further purification.

Kit Contents

Product Number	R6825-00	R6825-01	R6825-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind? DNA Mini Column	5	50	200
HiBind? RNA Mini Column	5	50	200
2 ml Collection Tubes	10	100	400
Glass Beads I (0.1-0.2mm)	1.5 g	60 g	240 g
Glass Beads II (0.4-0.6mm)	1.5 g	60 g	240 g
Buffer SLX	5 mL	25 mL	100 mL
HTR2 Reagent*	0.5 mL	3 mL	15 mL
SP2 Buffer	0.5 mL	3 mL	15 mL
Binding Buffer	3 mL	25 mL	100 mL
RNA Binding Buffer	5 mL	50 mL	200 mL
RWC Wash Buffer	5 mL	35 mL	120 mL
RNA Wash Buffer II	2 mL	20 mL	2x40 mL
DEPC Water	2 mL	20 mL	80 mL
Instruction Booklet	1	1	1

*HTR2 Reagent is a solid suspension and does not need to be heated for dissolution.

Storage and Stability

All components of the E.Z.N.A.? Soil RNA Mini 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 24 months from date of purchase.

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 80 mL (96%-100%) ethanol.
R6825-02	Add 160 mL (96%-100%) ethanol to each bottle.

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
- Water saturated phenol
- Chloroform

E.Z.N.A.® Soil RNA Mini Kit Protocol

1. **Weigh 200mg Glass Beads I and 200mg Glass Beads II in a 15 mL centrifuge tube, add 0.5g soil sample.**

2. **Add 400 µl Buffer SLX, 40 µl HTR2 Reagent** to the sample.

   Note: Shaking to resuspend HTR2 Reagent before used.

3. **Add 400 µl water-saturated phenol to the sample.** Vortex at maxi speed for 10 minutes. For the best result, beads mixer such as FastPrep-24 should be used.

4. **Add 400 µl Chloroform to the sample.** Vortex at maxi speed for 1 minute.

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

6. Carefully transfer 350 µl upper aqueous phase to a new 2 ml tube. **Add 0.1 volume**(35 µl) **of SP2 Buffer and equal volume** (350 µl) **of Binding Buffer to the sample.**

7. Apply the mixture sample from step 6 into a HiBind® DNA Mini Column inserted in a 2ml microfuge tubes. Centrifuge at 12,000 x g for 1 minutes.

8. Discard the HiBind® DNA Mini Column and **add equal volume of RNA Binding Buffer to the flow through**. Mix throughly by invert the tube 10-30 times.

9. Transfer 750 µl mixture sample from step 8 into a HiBind® RNA Mini Column inserted in a new 2 ml collection tube. Centrifuge at 12,000 x g for 1 minutes. Discard flow-through and re-use the collection tube.

10. **Repeat Step 9 with remainder sample,** discard the flow through and the collection tube**.**

11. Place the column into a new 2 ml collection tube. **Add 500 µl RWC Wash Buffer into the column.** Centrifuge at 12,000 x g for 1 minutes. Discard the flow through and re-use the collection tube.

12. Place the column into the same centrifuge tube from previous step. **Add 700 µl RNA Wash Buffer II into the column.** Centrifuge at 12,000 x g for 1 minutes. Discard the flow through and re-use the collection tube.

13. **Repeat Step 12 with a second 700 µl RNA Wash Buffer II**. Discard the flow through and re-use the centrifuge tube.

14. Place the column into same collection tube from previous step and centrifuge the empty column for 2 minute at 12,000 x g.

15. Place the HiBind® RNA Mini Column into a new 1.5 ml centrifuge tube.

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

17. Centrifuge at 12,000 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 II with 96-100% ethanol
	Sample stored incorrectly	Use fresh sample
Low RNA yield or no RNA eluted	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 4).
	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

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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