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Introduction

The E.Z.N.A.? Stool RNA Kit allows rapid and reliable isolation of high-quality total RNA from fresh and frozen stool samples. Up to 200 mg of stool samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid-binding properties of HiBind? matrix with the speed and versatility of spin column technology to eliminate humic acid, polysaccharides, phenolic compounds, and enzyme inhibitors from stool samples. Purified RNA is suitable for RT-PCR and Northern Blot.

Overview

If using the E.Z.N.A.? Stool RNA Kit for the first time, please read this booklet to become familiar with the procedures. Frozen or fresh stool samples are homogenized and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently Removed with chloroform. Binding conditions are then adjusted by adding RB Buffer and the sample is applied to a HiBind? RNA Column. Two rapid wash steps remove trace contaminants, and pure RNA is eluted with DEPC Water.

Storage and Stability

All components of the E.Z.N.A.? Stool RNA Kit should be stored at 22°C-25°C. 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.

Kit Contents

Product Number	R6828-00	R6828-01	R6828-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind? RNA Columns	5	50	200
2 mL Collection Tubes	15	150	600
Buffer RPL	12 mL	100 mL	2 x 200 mL
Buffer RB	5 mL	35 mL	125 mL
Glass Beads	1.2 g	12 g	45 g
RWC Wash Buffer	5 mL	50 mL	200 mL
RWB Wash Buffer	2 mL	12 mL	50 mL
DEPC Water	5 mL	20 mL	60 mL
Instruction Booklet	1	1	1

Before Starting

Materials to Be Provided by User

- Microcentrifuge capable of at least 10,000 x g
- Nuclease-free 2 mL tube or 1.5 mL microfuge tubes
- Water bath or heating block preset to 65°C
- Water saturated phenol
- Chloroform
- Absolute (96%-100%) ethanol

- 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.
- Dilute RWB Wash Buffer with absolute ethanol as follows and store at room temperature.

R6828-00	Add 8 mL absolute (96%-100%) ethanol.
R6828-01	Add 48 mL absolute (96%-100%) ethanol.
R6828-02	Add 200 mL absolute (96%-100%) ethanol

Stool RNA Protocol

1. Weigh up to 100-200 mg of stool sample in a 2 mL centrifuge tube containing 200 mg of Glass Beads and place the tube on ice.

   Note: If the sample is liquid, pipet 200µL of sample into the centrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a spatula to scrape the sample into the tube. Do not thaw the frozen sample until the Buffer RPL is added into the tube.

2. Add 500 µl Buffer RPL and vortex to mix well.

3. Add 500 µl Water Saturated Phenol, vortex mix well. Incubate at 65°C for 10 min.

4. Optional: For isolation of RNA from bacteria, Vortex at maxi speed for 3 minutes.

5. Add 500 µl Chloroform. Mix the sample throughly by voretxing the tube for 30 seconds.

6. Incubate the sample on ice for 5 minutes.

7. Centrifuge at full speed (<13,000 x g) in a microcentrifuge for 5 minute.

8. Carefully aspirate 500 µl supernatant to a new 2 mL microfuge tube (not supplied), making sure not to disturb the pellet or transfer any debris.

9. Add 500 µl Buffer RB and 500 µl absolute ethanol, vortex to mix well.

10. **Apply 750 ul of sample from step 9 to a HiBind? RNA Column assembled in a 2 mL collecting tube (supplied).** Centrifuge at full speed (>10,000 x g) for 30 seconds at room temperature. Discard flow-through liquid and re-use the collection tube in next step.

11. Repeat steps 10 to load the remaining of samples to the column. Centrifuge as above and discard the flow-through and the collection tube.

12. **Pipet 500µl RWC Wash Buffer into the column. Centrifuge as above and** Discards the flow-through. Re-use the collection tube in step 13.

13. Place the column into a new collection tube (provided). **Pipet 500 µl RWB Wash Buffer (prediluted with absolute ethanol) into the column**. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.

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

14. **Add another 500 µl RWB Wash Buffer to the column and centrifuge at 10,000 x g for 1 minutes.** Discard the flow-through and re-use the collection tube.

15. Place the HiBind? RNA Column into the same 2 ml collection tube. Centrifuge at full speed (≥12,000 x g) for 2 minutes to completely dry the membrane.

16. Elution of RNA: Place the HiBind? RNA column onto a 1.5ml RNase-free microtube and **add 30-50 µl DEPC Water directly onto the center of HiBind? RNA Column silica membrane**. Let the column sit at room temperature for 2 minutes and centrifuge at 10,000 x g for 1 minutes to elute RNA.

To determine the concentration and purity of RNA, measure absorbency at 260 nm and 280 nm in a spectrophotometer. 1 O.D. unit measured at 260 nm corresponds to 44 µg of RNA per ml. If it is necessary to dilute RNA sample, use a buffer with neutral pH. The ratio of A_{260}/A_{280} of pure nucleic acids is 2.0, while for pure protein it is approximately 0.6. A ratio of 1.8-2.1 corresponds to 90%-100% pure nucleic acid. (Phenol has an absorbency maximum at 275 nm and can interfere with spectrophotometric analysis of DNA or RNA. However, the E-Z 96? RNA technology eliminates the use of phenol and avoids this problem.) Store RNA samples at -70°C in water. Under such conditions RNA is stable for more than a year.

Stool RNA Protocol for Viral Detect

1. Suspend 0.5-1.0 ml or 0.5-1g stool in up to 5 ml of 0.89% NaCl.

2. Clarify the solution by spinning 4,000xg for 20 minutes.

3. Filter the supernatant using 0.22 µm filter.

4. Transfer 150 µl of the filtrate into a new 1.5 ml tube. Add 500 µl Buffer RB buffer and vortex to mix well.

   Note: *Remember to add 20 µl of 2-mercaptoethanol per 1 ml of Buffer RB Buffer before use.* Increase the amount of Buffer RB proportionally if the sample volume is larger than 150 µl.

   Stool, plasma, serum, urine and other body fluids often contain only very low number of cells and viral. In this case we recommend concentrating the sample to final volume of 200ul by Ultra filtrate.

5. Incubate at room temperature for 5-10 minutes. Briefly spin to collect any liquid from lid.

6. **Add 350 µl of absolute ethanol (room temperature, 96-100%) to the sample, mix throughly by vortexing at maxi speed for 30 seconds**.

7. **Apply the 700 µl of the mixture (including any precipitate) to a HiBind? RNA column assembled in a 2 ml collection tube (supplied)**. The maximum capacity of the HiBind? RNA Column is 800 µl. During the procedure, work carefully but quickly. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through.

8. Repeat step 7 until all the lysate has been loaded into the column and passed through the column.

9. **Place the column into a clean 2 ml collection tube (supplied)**, **and add 500 µl RWB Wash Buffer diluted with ethanol**. Centrifuge as above and

discard flow-through.

Note: RWB Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle or before starting on page 3 for directions.

10. Place the column into a new 2ml collection tube and add **500ul RWB Wash Buffer again. Centrifuge as above and discard the flow-through.**

11. Place the column back into the collection tube, centrifuge the empty column at full speed(no more than 14,000 x g) for 2 min to completely dry the HiBind? matrix.

12. Transfer the column to a clean 1.5 ml microfuge tube (not supplied) and add 30-50 µl DEPC Water (supplied) directly onto column matrix. Allow the column to incubate for 3 to 5 min at room temperature. Centrifuge at 10,000 x g for 1 min to elute RNA. Store Purified RNA at -70° C.

Troubleshooting Guide

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC Water to 70°C prior to elution. • Incubate column for 10 min with water prior to centrifugation.
	Column is overloaded	• Reduce quantity of starting material.
Clogged column	Incomplete disruption or lysis of plant tissue.	• Increase centrifugation time. • Reduce amount of starting material
Degraded RNA	Source	• Follow protocol closely, and work quickly.
	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	• Ensure RWB Wash Buffer has been diluted with 100% ethanol as indicated on bottle. • Diluted RWB Wash Buffer must be stored at room temperature.
Low Abs ratios	RNA diluted in acidic buffer or water	• DEPC Water is acidic and can dramatically lower Abs260 values. Use TE buffer (pH 8) to dilute RNA prior to spec analysis.