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Introduction

E.Z.N.A.™ Bacterial RNA Kit allows rapid and reliable isolation of high-quality total cellular RNA from a wide variety of bacterial species. Up to 1 x 10^9 Bacterial cells can be processed. The system combines the reversible nucleic acid-binding properties of Omega Bio-Tek's HiBind® matrix with the speed and versatility of the spin column technology to yield approximately 50 -100 µg of RNA. There are no organic extractions, thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel. Purified RNA has Abs260/Abs280 ratios of 1.8-2.0 and is suitable for the following applications:

- RT-PCR
- Northern Analysis
- Differential display
- Poly A+ RNA selection

Overview

If using the E.Z.N.A.™ Bacterial RNA Kit for the first time, please read this booklet to become familiar with the procedures. Bacterial cells are grown to log-phase and harvested. Bacterial cell walls are removed by lysozyme digestion. Following lysis, binding conditions are adjusted and the samples are applied to HiBind® RNA spin-columns. Two rapid wash steps remove trace salt and protein contaminants, and RNA is eluted in water or low ionic strength buffer. 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.™ Bacterial RNA Kit are stable for at least 24 months from the date of purchase when stored at 22°C-25°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer BRK. It is possible to dissolve such deposits by warming the solution at 37°C.

New This Edition

A heat incubation step has been added to reduce the amount of co-purification of DNA.

Kit Contents

Product Number	R6950-00	R6950-01	R6950-02
Purification	5 Preps	50 Preps	200 Preps
HiBind® RNA Mini column	5	50	200
2 ml Collection Tubes	15	150	600
BRK Lysis Buffer	2 ml	20 ml	80 ml
RNA Wash Buffer I	5 ml	50 ml	200 ml
RNA Wash Buffer II	5 ml	12 ml	50 ml
Glass Beads	200 mg	2.0 g	8.0 g
Lysozyme	8 mg	80 mg	4 x 80 mg
DEPC Water	1.5 ml	10 ml	40 ml
User Manual	1	1	1

*Buffer BRK contains a chaotropic salt. Use gloves and protective eyeware when handling with this solution.

Before Starting

Please take a few minutes to read this booklet thoroughly to become familiar with the protocol. Prepare all materials required before starting procedure to minimize RNA degradation

- Prepare a stock solution of **lysozyme** (provided) at **15 mg/ml** with **TE buffer** and aliquot into adequate portions. Store aliquots at -20°C.
- Bacterial should be harvested in log-phase growth.
- **β-mercaptoethanol (β-ME)** must be added to **Buffer BRK** before use. This mixture can be stored for 1 month at room temperature.
- Dilute **RNA Wash Buffer II Concentrate** with **ethanol** as follows and **store at room temperature**.

R6950-00	Add 20 ml absolute ethanol (96%-100%)
R6950-01	Add 48 ml absolute ethanol (96%-100%)
R6950-02	Add 200 ml absolute ethanol (96%-100%) per bottle

Bacterial RNA Spin Protocol

Materials supplied by users

- Tabletop microcentrifuge and RNase free 2.0 or 1.5 ml tubes.
- Absolute ethanol (96%-100%) - do not use other alcohols.
- Waterbath or Incubator set to 70°C

This method allows bacterial RNA isolation from up to 3 ml LB culture.

1. **Grow Bacteria in LB media to log phase. (Do not use overnight culture.)**

2. Harvest no more than 3 ml culture (< 5 x 10^8 bacteria) by centrifugation at 4,000-5000 x g for 5-10 min at 4°C.

3. Discard medium and resuspend cells in 100µl Lysozyme/TE Buffer**. Mix by vortexing at maxi speed for 30 seconds.**

   Note: The amount of enzyme required and/or the incubation time may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis**. For some bacteria, other enzymes may be more effective.**

4. **Incubate at 30°C for 10 minutes. Incubate on a shaker-incubator or vortex for 20 seconds for every 2 minutes during incubation.**

5. Add 350 µl BRK lysis buffer and 25-40 mg glass beads to the sample and vortex vigorously for 5 minutes. Centrifuge for 5 minutes at maximum speed in a micro-centrifuge.

   Note: Ensure β-mercaptoethanol (β-ME) is added to BRK Lysis Buffer (20 µl/ml) before use.

6. **Transfer 400µl of the supernatant into a new 1.5 ml centrifuge tube.**

7. **Incubate sample at 70°C for 5 minutes. Centrifuge at maximum speed (>13,000 x g) for 2 minutes.** Transfer the supernatant into a new 1.5 ml tube (not supplied)

8. Add 280 µl absolute ethanol (96-100%) to the lysate and mix well by vortexing at maxi speed for 15 seconds.

9. Apply sample, including any precipitate that may have formed, to a HiBind® RNA mini column inserted in a 2 ml collection tube. Centrifuge for 30 seconds at 8,000-10,000 x g. Reuse the collection tube for next step.

10. Add 400µl RNA Wash Buffer I to the column. Centrifuge at 10,000 x g for 2 minutes. Discard the flow-through and collection tube. If on-membrane DNase I digestion is desired, proceed to Step 11, otherwise go to Step 13.

11. **DNase I Digestion (Optional)**
    Since HiBind® RNA resin and spin-column technology actually removes most of DNA without the DNase I treatment, it is not necessary to do DNase digestion for most downstream applications. However, certain sensitive RNA applications might require further DNA removal. Follow the steps below for on-membrane DNase I digestion. (See DNase I manual, Product No. E1091 for detailed information.)

    a. For each HiBind® RNA column, prepare the DNase I digestion reaction mix as follows:

OBI DNase I Digestion Buffer	73.5 µl
RNase-free DNase I (20 Kunitz unites/µl)	1.5 µl
Total volume	75 µl

Note:

- **DNase I is very sensitive and is subject to physical denaturation; so do not vortex the DNase I mixture. Mix gently by inverting the tube. Prepare the fresh DNase I digestion mixture before RNA isolation.**
- **OBI DNase I digestion buffer is supplied with OBI RNase-free DNase I set**.
- **Standard DNase buffers are not compatible with on-membrane DNase I digestion.**

b. Dry column by spinning an additional 30 seconds, then pipette 75 µl of the DNase I digestion reaction mix directly onto the surface of the HiBind® RNA membrane in each column. Make sure to pipette the DNase I digestion mixture directly onto the membrane. DNase I digestion will not be complete if some of the mix sticks to the wall or the O-ring of the HiBind® RNA column.

c. Incubate at room temperature(25-30°C) for 15 minutes.

11. Place HiBind® RNA Mini column in a clean 2 ml collection tube, and add 500 µl RNA Wash Buffer I. **(If on-membrane DNase I digestion was performed in the previous step, allow wash buffer to soak column at least 5 minutes before proceeding).** Centrifuge as above and discard flow-through.

12. Place HiBind® RNA Mini-column in a new 2 ml collection tube (provided). **Add 500 µl RNA Wash Buffer II and spin for 30 seconds at 10,000 x g.** Discard flow-through and reuse the collection tube.

13. **Add 500 µl RNA Wash Buffer II to column and centrifuge for 30 seconds at 8,000-10,000 x g to wash again**. Discard the flow-through and reuse the collection tube.

14. Using the same collection tube, dry the column by spinning for 2 minutes at 8000-10,000 x g.

Note: Drying the HiBind® RNA Mini column is very important for removal of residual ethanol that will otherwise interfere with downstream applications.

15. Transfer HiBind® RNA Mini column to a new RNase free 1. 5 ml collection tube (not supplied) and add 50-100 µl DEPC water directly onto the HiBind® membrane. Centrifuge for 1 minute at 8,000-10,000 x g to elute. Repeat if the expected RNA yield is more than 60 µg.

Vacuum/Spin Protocol (V-Spin Column Only)

Carry out lysis, homogenization, and loading onto HiBind® RNA column as indicated in previous protocol (Steps 1-8). Instead of continuing with centrifugation, follow steps blow.

Note: Please read through previous section of this manual before using this protocol.

1. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® RNA V-Spin column to the manifold.
2. **Load the homogenized sample into HiBind® RNA V-spin column.**
3. Switch on vacuum source to draw the sample through the column.
4. **(Optional): Perform on-membrane DNase I digestion steps if sensitive downstream application is desired. (See Step 10, Pages 5-6, above)**
5. Wash the column by adding 500 µl **RNA Wash Buffer I**. Draw the wash buffer through the column by turning on the vacuum source.
6. Wash the column by adding 500 µl **RNA Wash Buffer II**. Draw the wash buffer through the column by turning on the vacuum source.
7. Insert the column into a **2 ml collection tube** and transfer the column to a micro centrifuge. Spin for 1 minute to dry the column.
8. Place the column in a clean 1.5 ml micro centrifuge tube and add 50-100µl DEPC water. Stand for 1-2 minute and centrifuge for 1 minute to elute RNA.

Quantification and Storage of 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 40 µg of RNA per ml. 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 1.8-2.0 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.N.A.™ Bacterial RNA Kit eliminates the use of phenol and avoids this problem.) Store RNA samples at -70°C in water. Under such conditions RNA prepared with the E.Z.N.A.™ system is stable for more than a year.

RNA Quality

It is highly recommended that RNA quality be determined prior to all analyses. The quality of RNA can be assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Several sharp bands should appear on the gel. These are the 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and possibly viral RNA bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling, or storage. RNA molecules less than 200 bases in length do not efficiently bind the HiBind® matrix.

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 at RT for 10 min with DEPC water prior to centrifugation.
	Column is overloaded	• Reduce amount of starting material.
	Bacterial cell wall is not completely removed	• Use longer incubation time for lysozyme digestion or add more lysozyme.
Clogged column	Incomplete disruption or lysis of bacterial.	• Use longer incubation time for lysozyme. Increase centrifugation time. • Reduce amount of starting material
Degraded RNA	Source	• Follow protocol closely, and work quickly. • Make sure that 2-mercaptoethanol is added to BRK Lysis Buffer
	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 Wash Buffer II has been diluted with 96%-100% ethanol as indicated on bottle. • Diluted Wash Buffer II must be stored at room temperature. • Repeat wash with Wash Buffer II.
DNA contamination	Co-purification of DNA	• Digest with RNase-free DNase I and inactivate at 75°C for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	• DEPC-treated water is acidic and can dramatically lower Abs260 values. Use TE buffer (pH 8) to dilute RNA prior to analysis.