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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 cell 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 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 GTC It is possible to dissolve such deposits by warming the solution at 37°C.

Kit Contents

Product Number	R6950-00	R6950-01	R6950-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® RNA Mini column	5	50	200
2 ml Collection Tubes	10	100	400
Buffer BRK	2 ml	20 ml	80 ml
RNA Wash Buffer I	5 ml	45 ml	2 x 90 ml
RNA Wash Buffer II	2 ml	12 ml	4 x 12 ml
Glass powder	200 mg	2.0 g	8.0 g
Lysozyme	8 mg	80 mg	4 x 80 mg
DEPC Water	1.5 ml	10 ml	20 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.
- Dilute RNA Wash Buffer II Concentrate with ethanol as follows and **store at room temperature**.

R6950-00	Add 8 ml absolute ethanol (96%-100%)
R6950-01	Add 48 ml absolute ethanol (96%-100%)
R6950-02	Add 48 ml absolute ethanol (96%-100%) to each bottle

E.Z.N.A.® Bacterial RNA Spin Protocol

Have the following reagents and supplies ready before beginning procedure:

- Tabletop microcentrifuge and RNase free 2.0 or 1.5 ml tubes.
- ethanol (70%) - do not use other alcohols.

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 20 seconds for every 2 minutes during incubation.**

5. Add 350 µl Buffer BRK/2-Me and 25-40 mg Glass Powder to the sample and vortex vigorously for 5 minutes. Centrifuge for 5 minutes at maximum speed (≥13,000 x g)in a micro-centrifuge.

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

6. Transfer 400 ul of the supernatant into a new 1.5 ml tube. Add 400 µl 70% ethanol to the lysate and mix well by pipetting.

7. Apply sample, including any precipitate that may have formed, to a HiBind® RNA mini column inserted in a 2 ml collection tube. Centrifuge for 30-60 seconds at 10,000 x g. Discard the liquid and reuse the collection tube for next step.

8. **Place column in the collection tube**, and add 300µl RNA Wash Buffer I. Centrifuge at 10,000 x g for 30-60 seconds at room temperature and discard flow-through. If on-membrane DNase I digestion is desired, proceed to step 9, otherwise go to step 10.

9. **DNase I digestion (Optional)**

Since HiBind® RNA resin and spin-column technology actually removes most of DNA without the DNase treatment, it is not necessary to do DNase digestion for most downstream applications. However, certain sensitive RNA applications might require further DNA removal. Following steps provide on-membrane DNase I digestion:(see DNase I cat.# E1091for detail 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 for physical denaturaion, so do not vortex this 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 set**.
- **Standard DNase buffers are not compatible with on-membrane DNase digestion.**

b. Pipet 75 µl of the DNase I digestion reaction mix directly onto the surface of HiBind® RNA resin in each column. Make sure to pipet the DNase I digestion mixture directly onto the membrane. DNase I digestion will not be complete if some of the mix stick to the wall or the O-ring of the HiBind® RNA column.

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

10. **Place column in a clean 2ml collection tube**, and add 500 µl RNA Wash Buffer I. **(If on-membrane DNase digestion was performed in the previous step, wait at least 5 minutes before proceeding).** Centrifuge at 10,000 x g for 30-60 seconds at room temperature and discard flow-through.

11. **Place column in the same 2ml collection tube**, and add 500µl RNA Wash Buffer II diluted with ethanol. Centrifuge at 10,000 x g for 30-60 seconds at room temperature and discard the flow-through. Reuse the collection tube in step 12.

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

12. Wash column with a second 500µl of RNA Wash Buffer II as in step 11. Centrifuge at 10,000 x g for 30-60 seconds at room temperature and discard the flow-through. Then with the collection tube empty, centrifuge the spin cartridge at 10,000 x g for **2 min** at room temperature to completely dry the HiBind® matrix.

13. **Elution of RNA.** Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 30-50µl of DEPC-treated water (supplied with kit). Make sure to add water directly onto column matrix. Centrifuge at 10,000 x g for 1 min at room temperature. A second elution may be necessary if the expected yield of RNA >30 µg.

Alternatively, RNA may be eluted with a greater volume of water. While additional elutions increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution. Pre-heating the water to 70°C before adding to column and incubating column 5 min at room temperature before centrifugation may increase yields.

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 from step 6 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 9, Pages 4-5, above)**
5. Wash the column by adding 400 µl **RNA Wash Buffer I**. Draw the wash buffer through the column by turning on the vacuum source.
6. Wash the column by adding 700 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 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 1 minute to elute RNA.

Quantitation 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.

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 long incubation time for lysozyme digestion or add more lysozyme.
Clogged column	Incomplete disruption or lysis of bacterial.	• Use long 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 Buffer GTC
	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.