Contents

Contents 1
Introduction 2
Overview 2
New in this edition 2
Storage and Stability 2
Kit Contents 3
Before Starting 3
Bacterial RNA Extraction Protocol 4
Vacuum/Spin protocol 6
Determination of Yield and Quality 7
Troubleshooting 8

Introduction

E.Z.N.A.™ Bacterial RNA Extraction Kits allow 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 HiBind™ matrix with the speed and versatility of spin column technology to yield approximately 50 -100 µg of DNA with an A_{260}/A_{280} ratio of 1.8-2.0. 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 procedure..Bacterial cells are grown to log-phase and harvested. Bacterial cell wall is removed by lysozyme digestion. Following lysis, binding conditions are adjusted and the sample applied to an HiBind® RNA spin-column. Two rapid wash steps remove trace salt and protein contaminants and finally 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.

New in this edition:

- On-column DNase I digestion protocol included. (Page 5)
- Optional vacuum-spin (for V-Spin column) protocol include.(Page 9)

Storage and Stability

All components of the E.Z.N.A.™ Bacterial RNA Kit are stable for at least 24 months from 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, though they do not interfere with overall performance.

Kit Contents

Product	R6850-00 R6950-00	R6850-01 R6950-01	R6850-02 R6950-02
Components			
HiBind™ RNA columns	5	50	200
Homogenizer Column	5	50	200
2 ml Collection Tubes	15	150	600
Buffer BRL	1.0 ml	5 ml	20 ml
BRK Lysis Buffer	2 ml	20 ml	80 ml
RNA Wash buffer I	5 ml	40 ml	160 ml
RNA Wash buffer II	5 ml	12 ml	50 ml
Lysozyme	5 mg	50 mg	4 x 50 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 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 to minimize RNA degradation

- Please read the entire booklet to become familiar with the E.Z.N.A.™ Bacterial RNA Extraction Kit procedure.
- Prepare a stock solution of lysozyme (provided) at 20 mg/ml and aliquot into adequate portions. Store aliquots at -20ºC.
- Dilute DNA Wash Buffer Concentrate with ethanol as follows and **store at room temperature**.
- Bacterial should be harvest in log-phase growth.
- If using Bacterial RNA Extraction kit for the first time, read this manual carefully.
- Prepare lysozyme stock solution by dissolve to 10mg/ml with BRL buffer.
- β-mercaptoethanol (β-ME) must be add to Buffer BRK before use.
- Dilute RNA wash buffer II with appropriate volume of absolute ethanol.
- All the centrifuge steps are performed in a microfuge at room temperature.

R6850-00, D6950-00	Add 20 ml absolute (96%-100%) ethanol
R6850-01, D6950-01	Add 48 ml absolute (96%-100%) ethanol
R685050-02, D6950-02	Add 200 ml absolute (96%-100%) ethanol/bottle

Bacterial RNA Isolation Protocol

Have the following reagents and supplies ready:

- Tabletop micro centrifuge and RNase free 1.5 ml tubes.
- β-mercaptoethanol (β-ME)
- DEPC dH_2O.
- Absolute ethanol - do not use other alcohols.
- Molecular Biology grade Lysozyme (20mg/ml in TE buffer).

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 by centrifugation at 4,000-5000 x g for 5-10 min at 4°C.

3. Discard medium and resuspend cells in 80µl BRL buffer. **Add 20 µl of 50mg/ml lysozyme followed by 10 min incubation at 30°C.**
   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

4. Add 350 µl BRK lysis buffer to the sample and vortex vigorously. If there is insoluble material in the lysis, centrifuge for 5 minutes at maximum speed in a micro centrifuge and use only the supernatant in subsequent steps.
   Note: Ensure β-mercaptoethanol (b-ME) is added to BRK lysis buffer.

5. **Transfer the cell lysate into a Homogenizer Column pre-inserted in 2 ml collection tube. Centrifuge at maximum speed for 5 minutes to homogenize the sample.**

6. **Discard the Homogenizer column and transfer the flow-through sample in a new 1.5 ml microtube.**

7. **Add 250 µl absolute ethanol (96-100%) to the lysate and mix well by vortexing.**

8. **Apply sample including any precipitate which may have formed, to HiBind RNA minicolumn sitting in a 2 ml collection tube. Centrifuge for 30 second at 8000-10000 x g.** Reuse the collection tube for next sep. If on-membrane DNase I digestion is desired, proceed step 9, otherwise go to step 9.

9. **DNase 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 denaturation, 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 I set**.
- **Standard DNase buffers are not compatible with on-membrane DNase I digestion.**

   b. Spin 30 seconds to dry the column, then pipette 75 µl of the DNase I digestion reaction mix directly onto the surface of HiBind® RNA resin 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 stick to the wall or the O-ring of the HiBind® RNA column.

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

9. **Place HiBind® RNA spin-column in a clean 2ml collection tube**, and add 700 µl RNA Wash Buffer I. **(If on-membrane DNase I digestion was performed in the previous step, wait at least 5 minutes before proceeding).** Centrifuge and discard flow-through.

10 **Assemble an HiBind® RNA spin-column in a new 2 ml collection tube (provided). Add 700 µl RNA wash buffer II onto column and spin for 30 seconds at 8000-10,000 x g to wash. Discard flow through and reuse the collect tube.**

11 **Optional: Add 500 µl RNA wash buffer II onto column and centrifuge for 30 seconds at 8000-10000 x g to wash again. Discard the flow through and reuse the collection tube.**

12 Assemble the column to the collection tube and spin for 5 minutes at 8000-10,000 x g to dry the column. **It is very important to dry the column since the residual ethanol will interfere with downstream application.**

13 **Transfer RNA HiBind column to a new RNase free 1. 5 ml collection tube (not supplied) and add 40-50 µl DEPC water directly onto HiBind membrane. Centrifuge for 1 minute at 8000-10,000 x g to elute. Repeat if the expected RNA yield is over 60 µg**.

Vacuum/Spin Protocol for Bacterial RNA Extraction (V-Spin

column only)

Carry out lysis, homogenization, and loading onto HiBind® RNA column as indicated previous protocols (Step 1-7). Instead of continuing with centrifugation, follow steps blow.

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

1. Prepare the vacuum manifold according to manufacturer's instruction and connect the HiBind® RNA V-Spin column to the manifold.
2. **Load the homogenized sample onto HiBind® RNA V-spin column.**
3. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
4. **(Optional): Perform on-membrane DNase I digestion steps if sensitive downstream application is desired.**
5. Wash the column by adding 750 µl **RNA wash buffer I**, draw the wash buffer through the column by turn on the vacuum source.
6. Wash the column by adding 500 µl **RNA wash buffer II**, draw the wash buffer through the column by turn on the vacuum source.
7. Assemble 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 RNase-free water. Stand for 1-2 minute and centrifuge 1 minute to elute RNA.

Quantization 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.® Plant 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, thus the method enriches high quality RNA.

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.
	Bacterial cell wall is not completely removed	• Use long incubation time for lyticase digestion or add more lyticase.
Clogged column	Incomplete disruption or lysis of bacterial.	• Use long incubation time for lyticase 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 BRL 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 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 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 spec analysis.