Ord.-No.: 12-6850

E.Z.N.A.® Bacterial RNA Kit

– Instruction Manual –

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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 A260/A280 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

Theory

The E.Z.N.A. ® Bacterial RNA Kits use the reversible binding properties of the HiBind® matrix, a new silica-based material. This is combined with the speed of mini-column spin technology. A specifically formulated high salt buffer system allows more than 100 µg of RNA molecules greater than 200 bases to bind to the matrix.
Bacterial cells are grown to logphase 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.

Kit Components

E.Z.N.A.® Bacterial RNA Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-6850-00	12-6850-01	12-6850-02
Components			
HiBind® Columns	5	50	200
Shredder Columns	5	50	200
2 ml Collection Tubes	15	150	600
BRL Buffer	1 ml	10 ml	40 ml
BRK Lysis Buffer	5 ml	30 ml	100 ml
Lysozyme	5 mg	50 mg	4 x 50 mg
RNA Wash buffer I	5 ml	40 ml	200 ml
RNA Wash buffer II	5 ml	24 ml	2 x 50 ml
DEPC-dH_2O	1.5 ml	10 ml	20 ml
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Storage and Stability

E.Z.N.A.® Bacterial RNA Kit components should be stored at room temperature (22 °C – 25 °C). All E.Z.N.A.® Bacterial RNA Kit components are stable for at least 12 months from the date of purchase when stored at 22-25 °C. During shipment crystals may form in the RB Buffer. Warm up to 37 °C to dissolve.

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

! Whenever working with RNA, always wear one-way gloves to minimize RNase contamination. Use only fresh RNase-free disposable plastic pipette tips when using the supplied reagents.

! Work carefully but as quickly as possible during the procedure.

! Under cool ambient conditions, crystals may form in the BRK Buffer. This is normal and the bottle should be warmed (37 °C) to dissolve the salt before use.

! 2-mercaptoethanol (ß-mercaptoethanol) is necessary for denaturing RNases and must be added to BRK Buffer before use. Add 20 µl of 2-mercaptoethanol to 1 ml of BRK Buffer. Prepare these mixtures just before use, if possible, however, they can be stored for 1 week at room temperature.

! Prepare lysozyme stock solution by dissolve to 20mg/ml with BRL buffer.

! RNA Wash Buffer II is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-6850-00	Add 20 ml 100% EtOH to 5 ml Wash Buffer II
Kit 12-6850-01	Add 96 ml 100% EtOH to 24 ml Wash Buffer II
Kit 12-6850-02	Add 200 ml 100% EtOH to 50 ml Wash Buffer II

Store diluted DNA Wash Buffer at room temperature.

! All steps must be carried out at room temperature (22 – 25 °C).

E.Z.N.A.® Bacterial RNA Isolation Protocol

Materials required, but not supplied:

- 2-Mercaptoethanol
- 100 % Ethanol
- 70 % Ethanol
- Sterile RNase-free pipet tips and centrifuge tubes

1. Homogenization and lysis

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

Harvest no more than 3 ml culture by centrifugation at 4,000-5000 x g for 5-10 min at 4°C. Discard medium and resuspend cells in 100µl BRL buffer. For gram$^-$ Bacteria add 5 µl of 20mg/ml lysozyme followed by 5 min incubation at 30°C. For gram$^+$ Bacteria add 50 µl of 20mg/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*

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.

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

Discard the Shredder column and transfer the flow-through sample in a new 1.5 ml microtube. Add 250 µl absolute ethanol (96-100%) to the lysate and mix well by vortexing.

2. Load and bind

Apply sample including any precipitate that may have formed, to HiBind RNA minicolumn sitting in a 2 ml collection tube. Centrifuge for 30 second at 8,000-10,000 x g. Reuse the collection tube for next step.

3. Wash I

Add 700 µl RNA Washbuffer I to the column and centrifuge the spin column / collection tube assembly for 30 sec at 10,000 x g. Place the spin column in a fresh 2 ml collection tube (supplied). Discard the flow-throw liquid and the used collection tube.

4. Wash II

Add 700 µl completed RNA Washbuffer II to the column and centrifuge the spin column / collection tube assembly for 30 sec at 10,000 x g. Discard the flow-through liquid. Repeat this wash step using the same collection tube and discard the flow-through liquid.

5. Dry (Important, do not skip this step!)

Place the HiBind® spin column containing your RNA in the collection tube used in step 5 and centrifuge for 2 min at 10,000 x g to dry the column matrix. This step is essential to remove ethanol from the column.

6. Elution

Place the HiBind® spin column (step 6) into a fresh 1.5 ml microcentrifuge tube. Add 40 - 50 µl (depending on the desired final concentration of RNA) sterile DEPC–dH_2O directly to the binding matrix in the spin column and centrifuge for 1 min at 10,000 x g to elute RNA.

A second elution may be necessary if the expected yield of RNA is >50 µg. Alternatively, RNA may be eluted with a higher volume of water. While additional elution increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution. Pre-heating DEPC-dH_2O to 70 °C before adding to the spin column and incubating the spin column for 5 min at room temperature before centrifugation may increase yield.

DNA Contamination

No RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR* or differential display) we suggest that you treat the eluted RNA with RNase-free DNase. Also for RT-PCR, use intron-spanning primers that allow easy identification of DNA-contamination. Using RNA as a template in a control PCR* reaction will also allow the detection of DNA contamination.

* PCR is covered by patents owned by F. Hoffmann-La Roche Ltd

Quantitation and storage of RNA

Determine the absorption of an appropriate dilution (10- to 50-fold) of the sample at 260 nm and then at 280 nm.

DEPC-water is slightly acidic and can dramatically lower absorption values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometer analysis.

One A_{260}-unit is about 40 µg RNA/ml. The RNA concentration is calculated as follows:

RNA conc. (µg /ml) = Absorption$_{260}$ × 40 × Dilution Factor

The ratio of $A_{260/280}$ is an indication of nucleic acid purity. Values higher than 1.8 indicates > 90% nucleic acid.

Phenol has an absorption maximum at 275 nm and can interfere with absorption readings 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 sterile DEPC-dH_2O. Under such conditions RNA prepared with the E.Z.N.A® system is stable for at least one year.

RNA Quality

It is highly recommended to determine the RNA quality prior to further applications. Denaturing agarose gel electrophoresis and ethidium bromide staining can best assess the quality of RNA. Two sharp bands should appear on the gel. These are the 28S and 18S ribosomal RNA bands. If these bands smear towards lower molecular weight RNA, then the RNA has undergone major degradation during preparation, handling, or storage.

Although RNA molecules less than 200 bases in length do not efficiently bind the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Ordering information

For RNA isolation from cells, tissues and blood:

E.Z.N.A.® Plant RNA Kit	12-6627-00	5 Preparations
	12-6627-01	50 Preparations
	12-6627-02	200 Preparations
E.Z.N.A.® Bacterial RNA Kit	12-6850-00	5 Preparations
	12-6850-01	50 Preparations
	12-6850-02	200 Preparations
E.Z.N.A.® Viral RNA Kit	12-6874-00	5 Preparations
	12-6874-01	50 Preparations
	12-6874-02	200 Preparations
E.Z.N.A.® Total RNA Kit (Safety-Line)	12-6834-00	5 Preparations
	12-6834-01	50 Preparations
	12-6834-02	200 Preparations
E.Z.N.A.® Total RNA Kit (Classic-Line)	12-6634-00	5 Preparations
	12-6634-01	50 Preparations
	12-6634-02	200 Preparations
E.Z.N.A.® Blood RNA Kit (Safety-Line)	12-6814-00	5 Preparations
	12-6814-01	50 Preparations
	12-6814-02	200 Preparations
E.Z.N.A.® Blood RNA Kit (Classic-Line)	12-6614-00	5 Preparations
	12-6614-01	50 Preparations
	12-6614-02	200 Preparations

Troubleshooting Tips

Problem	Likely 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.	• Completely disrupt sample in liquid nitrogen. • Increase centrifugation time. • Reduce amount of starting material
Precipitated RNA will not dissolve.	High nucleic acid and polysaccharide content.	• Reduce amount of starting material. Generally it is best to start with 50-100 mg at first. • To avoid RNA degradation, do not increase incubation time for resuspension.
Degraded RNA	Source	• Follow protocol closely, and work quickly. • Make sure that 2-mercaptoethanol is added to Buffer BRK.
	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 incubate at 37°C for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	• DEPC-treated water is acidic and can dramatically lower Abs_{260} values. Use TE buffer (pH 8) to dilute RNA prior to spec analysis.