BACTERIAL RNA PURIFICATION KIT

Product Manual

26-005.....5 preps
26-011.....50 preps
26-011B.....50 preps
P/N 03-293-5 REV. D

OMNI INTERNATIONAL

TABLE OF CONTENTS

INTRODUCTION

The Omni 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 the Omni RNA mini column matrix with the speed and versatility of the spin column technology to yield approximately 50 -100 µg of RNA. Purified RNA has A_{260}/A_{280} ratios of 1.8 - 2.0 and is suitable for downstream applications including:

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

OVERVIEW

If using the Omni 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 Omni RNA Mini column. Two rapid wash steps remove trace salt and protein contaminants then 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.

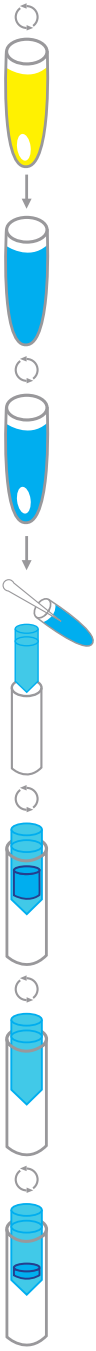
KIT COMPONENTS

Product Number	26-005	26-011	26-011B
Purification	5 Preps	50 Preps	50 Preps
Omni RNA Mini column	5	50	50
2 mL Collection Tubes	15	150	150
MRLB Lysis Buffer	5 mL	40 mL	40 mL
RW1 Buffer	5 mL	50 mL	50 mL
RW2 Buffer	5 mL	12 mL	12 mL
Lysozyme	8 mg	80 mg	80 mg
DEPC Water	1.5 mL	20 mL	20 mL
2 mL bead kits 0.5 mm glass	5		50
Antifoam	1 mL		1 mL
User Manual	✓	✓	✓

STORAGE AND STABILITY

Kit components can be stored at 22°C-25°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer MRLB. It is possible to dissolve such deposits by warming the solution at 37°C.

ILLUSTRATED PROTOCOLS



Pellet the bacterial cells. Add Lysozyme to remove the cell wall

Lyse and homogenize

Add Ethanol to adjust the binding conditions

Bind RNA

Wash Column x 2

Dry the column

Elute RNA

BEFORE STARTING

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

Materials supplied by users:

- Tabletop microcentrifuge
- Nuclease-free 1.5 mL micro-centrifuge tubes.
- Absolute ethanol (96%-100%)
- Waterbath or Incubator capable of 70°C
- Tris-EDTA buffer (TE Buffer - 10mM Tris-HCL, 0.1mM EDTA, pH 8.0)
- β-mercaptoethanol (βME)
- Vortexer or Bead Mill Homogenizer

PREPARING REAGENTS

Prepare all materials required before starting procedure to minimize RNA degradation

Dilute RW2 Buffer concentrate with ethanol as follows and store at room temperature.

Kit	100% Ethanol to be added
26-005	20 mL
26-011 and 26-011B	48 mL

- Prepare a stock solution of lysozyme (provided) at 15 mg/mL with Tris-EDTA buffer and aliquot into adequate portions. Store aliquots at -20°C
- Bacteria should be harvested in log-phase growth.
- β-mercaptoethanol (βME) must be added to MRLB Buffer before use (20 µL βME per 1 mL MRLB). This mixture can be stored for 1 month at room temperature.
- Prepare RW2 buffer as instructed in "Preparing Reagents" section
- Heat water bath or incubator to 70°C
- Pre-heat EB buffer to 70°C

BACTERIAL RNA SPIN PROTOCOL

1. Grow Bacteria in LB media to log phase. (Do not use overnight culture.)
2. Harvest no more than 3 mL culture (< 5 x 10^9 bacteria) by centrifugation at 4,000-5000 x g for 5-10 min at 4°C.
3. Aspirate and discard media
4. Re-suspend cells in 100 µL Lysozyme/TE Buffer. Mix by vortexing.

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.

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

26-011 - Cell Digestion

6. Add 350 uL MRLB buffer. Transfer entire contents to a nuclease free 1.5 mL microcentrifuge tube (not provided). Vortex for 10 seconds..

Note: Ensure βME is added to MRLB (20 µL/ mL)

26-011B - Cell Homogenization

6. Add 350 uL MRLB buffer and 10 uL Antifoam reagent. Transfer entire contents to a 2 mL tube containing 0.5 mm glass beads. Homogenize cells on a bead mill for 30-60 seconds or with continuous vortexing on high speed for 5 minutes.

Note: Bead Mill speed/power and time settings should be adjusted based on the equipment manufacturer's recommendations for the specific sample type.

7. Centrifuge for 5 minutes at 10,000 x g in a micro-centrifuge.
8. Transfer 400 µL of the supernatant into a new nuclease-free 1.5 mL micro-centrifuge tube (not supplied).
9. Incubate sample at 70°C for 5 minutes.
10. Centrifuge at 10,000 x g for 2 minutes.
11. Transfer the supernatant into a new nuclease-free 1.5 mL microcentrifuge tube (not supplied)

12. Add 280 µL absolute ethanol (96-100%) to the lysate and mix well by vortexing for 15 seconds.
13. Apply sample, including any precipitate that may have formed, to an Omni RNA mini column inserted in a 2 mL collection tube.
14. Centrifuge for 30 seconds at 10,000 x g. Reuse the collection tube for next step.
15. Add 400 µL RW1 Buffer to the column.
16. Centrifuge at 10,000 x g for 2 minutes. Discard the flow-through and collection tube.
17. Place Column on new collection tube.
18. Add 500 µL RW2 Buffer to column.
19. Centrifuge for 30 seconds at 10,000 x g to wash again. Discard the flow-through and reuse the collection tube.
20. Using the same collection tube, dry the column by spinning for 2 minutes at 10,000 x g.

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

21. Transfer the Omni 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 Omni membrane.

Note: Centrifuge for 1 min at 10,000 x g. A second elution may be necessary if the expected yield of RNA is greater than 60 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.

22. Store RNA sample at -70°C

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. RNA prepared with the Omni system is stable for more than a year at -70°C.

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 Omni matrix.

TROUBLE SHOOTING GUIDE

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the column	• Repeat elutions • Preheat DEPC water to 70° prior to elution • Incubate column for 5 min with water prior to centrifugation.
	Column is overloaded	• Reduce quantity of starting material
	Bacterial cell wall is not completely removed	• Use longer incubation time for lysozyme digestion or add more lysozyme. • Increase mechanical homogenization force or time.
Clogged Column	Incomplete disruption or lysis of bacterial	• Use longer incubation time for lysozyme. • Increase mechanical homogenization force or time. • Increase centrifugation time • Reduce amount of starting material.
Problems in downstream applications	Salt carry-over during elution	• Ensure RW2 buffer concentration has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • RW2 buffer must be stored and used at room temperature. • Repeat wash with RW2 buffer.
Degraded RNA	Source	• Use only freshly harvested cells • Do not store cells prior to extraction unless they are lysed with buffer RLB first. • Follow protocol closely and work quickly • Make sure that 2-mercaptoethanol is added to MRLB Buffer
	RNase Contamination	• Ensure not to introduce RNase during the procedure • Check buffers for RNase contamination
DNA Contamination	Co-Purification of DNA	• Digest with RNase-free DNase after elution and inactive at 75°C for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or DEPC water.	• DEPC treated water is acidic and can dramtically lower A 260 values. Use TE buffer at ph 8.0 to dilute RNA prior to spectrophotometric analysis.

NOTES

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