YEAST RNA PURIFICATION KIT

Product Manual

26-006...............5 preps
26-012...............50 preps
26-012B...........50 preps
P/N 03-293-6 REV. E

TABLE OF CONTENTS

1. Introduction..Pg1

2. Overview...Pg1

3. Kit Components Pg2

4. Storage and Stability Pg2

5. Illustrated Protocols Pg3

6. Before Starting...Pg4

7. Preparing Reagents..Pg5

8. Yeast RNA Spin Protocol..Pg5

9. Quantification and Storage of RNA......................................Pg7

10. RNA Quality Pg7

11. Trouble Shooting Guide Pg8

INTRODUCTION

The Omni Yeast RNA Kit allows convenient isolation of high-quality total RNA from a wide variety of yeast species. Up to 2 x 10^7 log-phase cultured yeast cells can be processed. The system combines the reversible nucleic acid-binding properties of Omni mini column matrix with the speed and versatility of spin column technology to yield approximately 30 µg of RNA, with an A_{260}/A_{280} ratio of 1.7-1.9. Purified RNA is suitable for downstream applications such as RT-PCR, DD-PCR, and hybridization techniques. There are no organic extractions, thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

OVERVIEW

If working with RNA for the first time, please read this booklet to become familiar with the procedure. Yeast cells are grown to log-phase and spheroblasts are subsequently prepared using SE Buffer and lyticase. Following lysis, binding conditions are adjusted and the sample is applied to a Omni RNA mini spin-column. Two rapid wash steps remove trace salt and protein contaminants, and RNA is eluted in DEPC-treated water. Purified RNA can be directly used in downstream applications without need for further purification.

BINDING CAPACITY

Each Omni RNA Mini Column can bind up to 100 ug Total RNA. Use no more than 3 ml log phase yeast culture.

KIT COMPONENTS

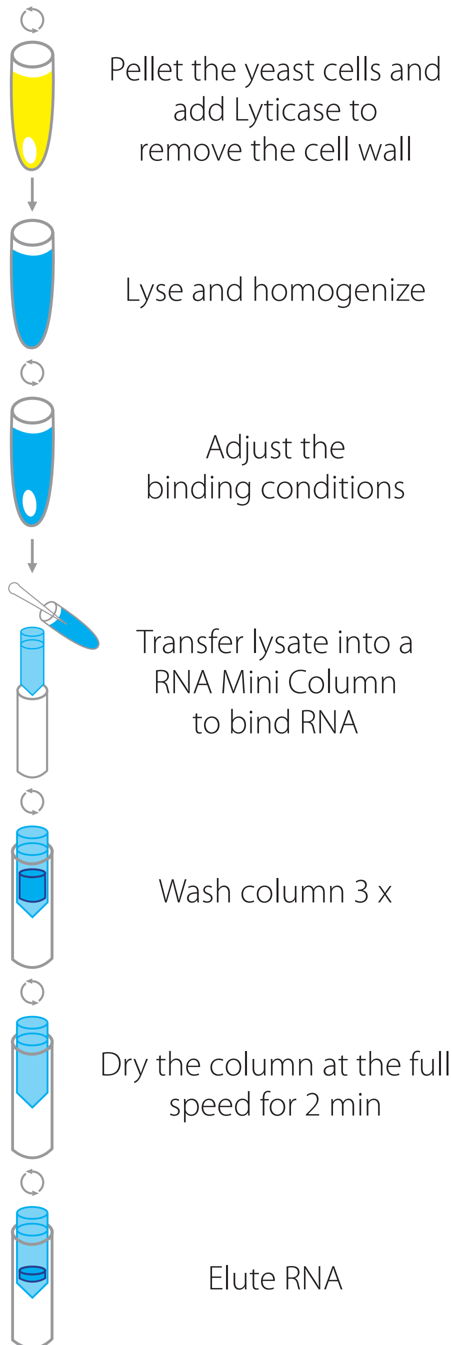
Product Number	26-006	26-012	26-012B
Purification	5 Preps	50 Preps	50 Preps
Omni RNA Mini Columns	5	50	50
2 mL Collection Tubes	15	150	150
LD Buffer	15 mL	120 mL	120 mL
MRLB Buffer	5 mL	40 mL	40 mL
RW1 Buffer	5 mL	50 mL	50 mL
RW2 Buffer	5 mL	12 mL	12 mL
Lyticase	1,400 units	14,000 units	14,000 units
DEPC - water	1.5 mL	20 mL	20 mL
2 mL bead kit 0.5 mm glass	5		50
Antifoam	1 mL		1 mL
User Manual	✓	✓	✓

*MRLB Buffer contains a chaotropic salt. Use gloves and protective eyewear when handling this solution.

STORAGE AND STABILITY

All components are stored at 22°C-25°C. During shipment or storage in cool ambient conditions, precipitates may form in MRLB Buffer. These precipitates should be dissolved by warming the solution at 37°C with gently shaking.

ILLUSTRATED PROTOCOLS



BEFORE STARTING

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

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

Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.

Prepare all materials required before starting the procedure to minimize RNA degradation.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x g
- Swing-bucket centrifuge
- Waterbath, oven, or heatblock capable of 30°C
- RNase-free pipette tips
- RNase-free 1.5 or 2 mL microcentrifuge tubes
- RNase-free 15 mL centrifuge tubes
- 100% ethanol
- 70% ethanol
- β mercaptoethanol (βME)
- Vortexer or Bead Mill Homogenizer

PREPARING REAGENTS

1. Prepare a lyticase stock solution (5,000 U/mL) as follows, aliquot, and store at -20°C.

Kit	LD Buffer to be added
26-006	220 µL
26-012 and 26-012B	2.2 mL

2. Under cool ambient ambient conditions, crystals may form in Buffer MRLB. This is normal and the bottle should be warmed to redissolve the salt.

3. β mercaptoethanol (βME) is key in denaturing RNases and must be added to an aliquot of LD Buffer before use. Add 20 µl of 2-mercaptoethanol per 1 ml of LD Buffer. This mixture can be stored for 1 month at room temperature.

4. Dilute RW2 Buffer with 100% ethanol as follows and store at room temperature.

Kit	Ethanol to be added
26-006	20 mL
26-012 and 26-012B	48 mL

OMNI YEAST RNA KIT - SPIN PROTOCOL

Prepare Lyticase, MRLB Buffer, and RW2 Buffer according to the "Preparing Reagents" section.

Heat waterbath, oven, or heat block to 30°C.

While the Omni RNA Mini Columns can bind up to 100 µg RNA, for effective purification, use no more that $5x10^7$ log-phase yeast cells. For ***S. cerevisiae*** grown in YPD media, an OD_{600} of 1.0 corresponds to approximately $2x10^7$ cells per mL.

1. Collect no more than 5×10^7 yeast cells in a 15 ml tube by centrifuging for 5 min at 1000 x g at 4°C. NOTE: Use only freshly harvested cells for preparation of spheroblasts.

2. Aspirate and discard media, resuspend cells in 480 µL LD Buffer/ βME and 40 µL lyticase solution. Resuspend the pellet by vortexing at max speed for 1 minute. Complete resuspension of cell pellet is vital for obtaining good yields. Incubate at 30°C for at least 30 min.

Note: Remember to add 10 µl of 2-mercaptoethanol per 1 ml of LD Buffer before use. This mixture can be made and stored at room temperature for 1 week.

3. Pellet spheroblasts by centrifuging 5 min at 400 x g at room temperature. Carefully aspirate and discard supernatant. Incomplete removal of supernatant will prevent complete lysis of spheroblasts in the next step.

26-012 - Cell Digestion

4. Add 350 µL MRLB buffer/2-mercaptoethanol to spheroblasts and transfer entire contents to a nuclease free 1.5 mL microcentrifuge tube (not provided). Vortex for 10 seconds.

26-012B - Cell Homogenization

4. Add 350 µL MRLB buffer and 10 µL 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

5. Centrifuge at 10,000 x g for 3 min. Transfer the supernatant into a new centrifuge tube.
6. Add an equal volume 70% ethanol to the lysate and mix thoroughly by pipetting or vortexing at max speed for 15 seconds. A white precipitate may form upon addition of ethanol; it will not interfere with the procedure. Do not centrifuge the tube.
7. Apply the entire sample (around 700 µl) to a Omni RNA Mini column assembled in a 2 ml collection tube (supplied). Centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and re-use collection tube in Step 8 and Step 9.
8. Wash the sample by adding 500 µl of RW1 buffer to the column. Centrifuge at 10,000 x g for 15 seconds at room temperature. Discard the flow-through and re-use the collection tube.
9. Add 500 µl RW2 Buffer diluted with ethanol. Centrifuge at 10,000 x g for 15 seconds and discard flow-through. Re-use the collection tube in next step.

Note: RW2 Buffer Concentrate must be diluted with absolute ethanol before use. Refer to Page 4 or to label on bottle for directions.

10. Wash sample with an additional 500 µl of RW2 Buffer, centrifuge and discard flow-through as in preceding step.
11. Using the same collection tube, centrifuge the spin cartridge at 10,000 x g for 1 min at full speed to completely dry the column matrix.
12. Elution of RNA. Transfer the column to a clean 1.5 ml RNase free microcentrifuge tube (not supplied with kit) and elute the RNA with 50-100 µl of DEPC-treated water (supplied with kit). Make sure to add water directly onto column matrix. Centrifuge 1 min at maximum speed. A second elution may be necessary if the expected yield of RNA is greater than 50 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.

QUANTITATION AND STORAGE OF RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm in a spectrophotomer. 1 O.D. unit measured at 260 nm corresponds to 40 µg of RNA per ml. DEPC-water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometeric analysis. 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. Store RNA samples at -70°C in water. Under such conditions RNA prepared with the Omni system is stable for more than a year.

RNA QUALITY

It is highly recommend that RNA quality be determined prior to all analyses. The quality of RNA can best be assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Two sharp bands should appear on the gel. There are the 28S and 18S ribosomal RNA bands. If these band smear toward lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling, or storage.

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 5min with water prior to centrifugation.
	Column is overloaded	• Reduce quantity of starting material
Clogged Column	Incomplete lysis	• Extend incubation with Lyticase • Increase Lyticase to 200 units per 10^7 cells. • Reduce amount of starting material. • Increase bead beating force or time
Degraded RNA	Source	• Use only freshly harvested cells • Do not store cells prior to extraction unless they are lysed with buffer MRLB first. • Follow protocol closely and work quickly • Make sure that βME is added to MRLB Lysis Buffer
	RNase Contamination	• Ensure not to introduce RNase during the procedure • Check buffers for RNase contamination
Problems in downstream applications	Salt carry-over during elution	• Ensure RW2 buffer concentrate has been diluted with 4 volumes of 100% ethanol as indicated on botltle. • RW2 buffer must be stored and used at room temperature. • Repeat with RW2 buffer.
DNA Contamination		• Digest with RNase free Dnase after elution and inactive at 75° for 5min
Low Abs ratio	RNA diluted in acidic buffer or DEPC water.	• DEPC treated water is acidic and can dramatically lower A260 values. Use TE buffer at pH 8.0 to dilute RNA prior to spectrophotometric analysis.

NOTES

OMNI
INTERNATIONAL

935-C Cobb Place Blvd
Kennesaw, GA 30144
Phone: 800.776.4431
Fax: 770. 421.0206
www.omni-inc.com