TISSUE RNA PURIFICATION KIT

Product Manual

26-004.....5 preps
26-010.....50 preps
26-010B.....50 preps
P/N 03-293-4 REV. E

OMNI INTERNATIONAL

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INTRODUCTION

The Omni Tissue RNA Kit is designed for isolation of total RNA from animal tissues. The Omni Tissue RNA Kit allows simultaneous processing of multiple tissue samples in less than 60 minutes. This procedure completely removes contaminants and enzyme inhibitors making RNA isolation fast, convenient, and reliable.

OVERVIEW

The Omni Tissue RNA Kit uses the reversible binding properties of the Omni RNA Mini Column matrix, a silica-based material. The sample is lysed first under highly denaturing buffer conditions so that RNase's are inactivated and the intact Tissue RNA is protected from degradation. Cell debris is pelleted by centrifugation. After adding ethanol to the cleared lysate, the sample is loaded into the Omni RNA Mini column. With a brief centrifugation step, the samples pass through the column and the RNA binds to the Omni RNA Mini Column matrix. Trace DNA that may co-purify with RNA can be removed by an optional DNase treatment step on the RNA spin column. After two wash steps, purified total RNA is eluted with RNase-free water.

KIT COMPONENTS

Product Number	26-004	26-010	26-010B
Purifications	5	50	50
Omni RNA Mini Columns	5	50	50
2 mL Collection Tubes	15	150	150
RLB Buffer	5 mL	40 mL	40 mL
RW1 Buffer	5 mL	50 mL	50 mL
RW2 Buffer	5 mL	12 mL	12 mL
DEPC Water	1.5 mL	20 mL	20 mL
2 mL bead kit 2.8 mm ceramic	5		50
Antifoam	1 mL		1 mL
Instruction Manual	✓	✓	✓

STORAGE AND STABILITY

All components in the Omni Tissue RNA Kit can be stored at room temperature for 12 months. During shipping and storage, precipitate may form in the RLB Buffer. Simply warm to 37° C to dissolve.

ILLUSTRATED PROTOCOLS

Lyse and homogenize tissues

Adjust the binding conditions

Transfer lysate into a RNA Mini Column to bind RNA

Wash column x 3

Dry the column

Elute RNA

BEFORE STARTING

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Prepare all of the materials required before starting to minimize RNA degradation.

Materials supplied by user

- 96-100% ethanol
- RNase-Free DNase I (optional)
- 2-Mercaptoethanol (βME)
- RNase-free filter pipette tips
- Bead Mill Homogenizer or Vortexer
- Centrifuge capable of 10,000 x g
- Water bath or heat block preset at 55°C
- Disposable gloves
- 70% ethanol in DEPC treated water

Although the binding capacity of the Omni RNA mini column is around 100 µg, the maximum amount of starting material depends on the type of tissue being processed and the corresponding RNA content.
It is essential to begin with the correct amount of tissue to get optimal RNA yield and purity with the Omni RNA Mini Column. For the first time user, we recommend to use less than 10 mg of tissue. Depending on the yield and purity obtained, it may be possible to increase the starting material to 30 mg.

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- During the procedure work carefully but quickly.
- Under cool ambient conditions, crystals may form in RLB Buffer. This is normal and the bottle should be warmed to redissolve the salt.
- 2-mercaptoethanol (βME) is key in denaturing RNase's and must be added to an aliquot of RLB Buffer before use. Add 20 µl of 2-mercaptoethanol per 1 mL of RLB Buffer. This mixture can be stored for 1 week at room temperature.
- All centrifugation steps must be carried out at 22°C-25°C

Note: Equilibrate samples and RLB buffer to room temperature before start. All steps must be carried out at room temperature. Work quickly, but carefully.

PREPARING REAGENTS

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

Kit	100% ethanol to be added
26-004	20 mL
26-010 and 26-010B	48 mL

OMNI TISSUE RNA PURIFICATION KIT DIRECTIONS

26-010 - Rotor Stator or Manual Tissue Disruption

1. Excise the tissue sample from the animal or from storage.
2. Weigh 10-30 mg of tissue

   A. Mortar and Pestle processing - Freeze sample in liquid nitrogen. Grind sample to fine powder under liquid nitrogen. Transfer powder to a clean nuclease free 1.5 mL microcentrifuge tube (not provided). Add appropriate amount of RLB Buffer/βME from table below.

   B. Rotor-Stator Homogenization - Transfer sample to nuclease free 2-5 mL tube (not provided). Add appropriate amount of RLB Buffer/βME from table below. Process sample until a full homogenate is achieved. Most tissues can be fully homogenized in less than 1 minute. Plastic Omni-Tip disposable generator probes (Cat# 30750H) are recommended for this process.

26-010B - Bead Mill Homogenization

1. Excise the tissue sample from the animal or from storage
2. Weigh 10-30 mg of tissue and place into a 2 mL tube containing 2.8 mm ceramic beads.
3. Add appropriate amount of RLB Buffer/βME from table below and 10 uL antifoam reagent.
4. Dissociate tissues on a bead mill. If a bead mill is not available the tissue can be processed on a vortexer at maximum speed for 10 minutes.

Amount of Tissue	Amount of RLB Buffer
≤ 15 mg	350 µL
20 -30 mg	700 µL

Note: Bead Mill speed/power and time settings should be adjusted based on the equipment manufacturer's recommendations for the specific sample type. When using mechanical methods of homogenization care must be taken to not over process as this could lead to RNA shearing.

5. Centrifuge at 10,000 x g for 5 minutes to pellet debris.
6. Transfer the cleared supernatant to a nuclease-free 1.5 mL microcentrifuge tube (Not provided)

Note: In some preparations, a fatty upper layer will form after centrifugation. Transfer of any of this layer may reduce RNA yield or clog the column.

7. Add 1 volume of 70% ethanol. Vortex for 10 seconds to mix thoroughly. Do not centrifuge.

Note: A precipitate may form at this point. This will not interfere with RNA purification. If any sample has lost its volume during homogenization, adjust the volume of ethanol accordingly.

8. Insert an Omni RNA Mini Column into a 2 mL Collection Tube.
9. Carefully transfer 700 µL of sample from step 7 (including any precipitate) to the Omni RNA Mini Column.
10. Spin at 10,000 x g for 1 minute at room temperature. Discard the flow-through and re-use the collection tube.
11. Repeat steps 9 and 10 until the entire sample has been transferred.
12. Pipet 500 µL of RW1 Buffer into the column.
13. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through and collection tube.
14. Place the column into a new collection tube (provided). Pipet 500 µL of RW2 Buffer into the column.

Note: RW2 Buffer must be diluted with absolute ethanol before use. Refer to the label on the bottle for instruction.

15. Centrifuge at 10,000 x g for 1 minute.
16. Discard the flow-through and re-use the collection tube.
17. Add another 500 µL of RW2 Buffer to the column
18. Centrifuge at 10,000 x g for 1 minute. Discard the flow-through and reuse collection tube.

19. Centrifuge at 10,000 x g for 2 minutes to completely dry the column.
20. Elution of RNA: Place the Omni RNA Mini Column onto a 1.5 mL RNase-free microcentrifuge tube and add 50 µL of DEPC-treated water directly onto the center of Omni RNA Mini Column. Centrifuge at 10,000 x g for 2 minutes to elute the RNA.
21. If the expected RNA yield is greater than 30 µg, repeat the elution step as described with a second volume of DEPC-treated water, collect the second elution with the same collection tube. To obtain a higher concentration of RNA, the second elution can be performed using the first elute (from step 16). Heating the DEPC water to 70° C before adding to the column and incubating the column after the addition of DEPC water for 5 minutes at room temperature can increase RNA yield as well.

INTEGRITY OF RNA

Run a denatured gel to determine the integrity and the size distribution of the RNA. The respective ribosomal RNA bands should appear as sharp and clear bands on the gel. If the ribosomal RNA bands in a given lane are not sharp and it shows smearing towards smaller sized RNA, it is very likely that the isolated RNA suffered major degradation during isolation procedure

QUANTIFICATION AND STORAGE OF RNA

To determine the concentration and purity of RNA, measure the absorbency at 260nm and 280nm in a spectrophotometer. One OD unit measured at 260nm 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 spectrophotometric analysis.
The A_{260} /A_{280} ratio of pure nucleic acids is 2.0, while for pure protein it is approximately 0.6. A ratio of 1.8-2.1 corresponds to 90%-100% pure nucleic acid. Store RNA samples at -80°C. Under such conditions RNA is stable for more than a year.

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
Clogged Column	Incomplete homogenization	• Completely homogenize sample • Increase configuration time • Reduce amount of starting material.
Degraded RNA	Source	• Do not freeze and thaw sample more than once • Follow protocol closely and work quickly.
	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	Co-purification of DNA	• Digest with RNase free Dnase after elution and inactive at 65°C for 5min in the presence of EDTA.
Low Abs ratio	RNA diluted in acidic buffer or water.	• DEPC water is acidic

NOTES

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