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Introduction

The EZgene™ Tissue RNA kit provides an easy and fast method for isolating total RNA from tissues, cultured cells within 30 min. Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNase I treatment (See detail in the protocol) when it is necessary.

Storage and Stability

All components can be stored at room temperature. All kit components are guaranteed for 1 year from the date of purchasing.

Kit Contents

Catalog#	R6311-01	R6311-02
Buffer LY	30 mL	135 mL
Buffer RB	30 mL	135 mL
RNA Wash Buffer	12 mL	50 mL
DEPC-Treated ddH_2O	10 mL	30 mL
RNA Columns	50	250
User Menu	1	1

Important

- ☑ Add 1% volume of β-mercaptoethanol to Buffer LY before use and store at 4 °C.
- ☑ Add 48 mL (R6311-01) or 200 mL (R6311-02) 100% ethanol to RNA Wash Buffer before use.

Table I: Recommend amounts of cells or tissues per preparation

Sample	Max Tissue or Cell Mass per prep	Total RNA Yield (μg)
Liver	30 mg	130
Kidney	20 mg	45
Muscle	80 mg	50
Spleen	15 mg	80
Heart	100 mg	50
Brain	120 mg	80
Lung	100 mg	70
Pancreas	30 mg	100
Tomato Leaves	200 mg	30
HeLa Cells	$5x10^6$	120

Protocol for Extracting Total RNA from Cultured Cells

1. Cell preparation:
   a. For total RNA extraction from suspension cultured cells, collect cells by centrifuging at 300 x g for 5 min at 4°C. Discard the supernatant and proceed to step 2 immediately.
   b. For total RNA extraction from adherent cultured cells, remove the culture medium and immediately proceed to step 2.

2. Add **500 µL Buffer LY** to the cell pellet or directly into the well (for adherent cells).

   Ensure that ß-mercaptoethanol has been added before use.

3. Homogenize the lysate by vortexing vigorously or repeated pipetting.

4. Add **1/2 volume 100% ethanol** into the lysate (for example: 250 µL 100% ethanol for 500 µL lysate) and pipet 5 times to mix the solution. Vortex briefly if any precipitations.

5. Transfer the solution into a RNA column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.

6. Add **500 µL Buffer RB** to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.

7. **Optional:** Add **50 µL DNase I (5 U, RNase-free) solution** onto the center of the column and incubate at room temperature for 15 min. Add **500 µL Buffer RB** onto the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through. Add **500 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through

8. Add **500 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through and reuse the collection tube.

9. Centrifuge the empty column, with the lid open, at 12,000 rpm for 1 min.
   Note: Residual ethanol will be removed more effectively with the column lid open.

10. Transfer the column to a RNase-free 1.5 mL tube and add **50-100 µL DEPC-treated water** to the column. Centrifuge at 12,000 rpm for 1 min. The RNA is in the flow-through liquid. Store RNA at -20 °C.

Note: It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. An A_{260}/A_{280} ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

Protocol for Extracting Total RNA from Animal Tissue

1. Quickly weigh an appropriate tissue mass according to Table 1 (Page 3) and transfer the tissue into a 1.5 mL tube containing **500 µL Buffer LY**. Homogenize the tissue by a rotor starter or an ultrasonic homogenizer on ice.

   Ensure that β-mercaptoethanol has been added before use. Use of too much tissue per preparation will cause genomic DNA contamination.

2. Centrifuge the lysate at 12,000 rpm for 2 min at room temperature and transfer the cleared lysate to a clean 1.5 mL tube.

3. Add **0.5 volume 100% ethanol** to the lysate (for example: 250 µL 100% ethanol for 500 µL lysate).

4. Transfer the solution into a RNA column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.

5. Add **500 µL Buffer RB** to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through and reuse the collection tube.

6. **Optional:** Add **50 µL DNase I** (5U RNase-free) solution onto the middle of the column and incubate at room temperature for 15 min. Then add **500 µL Buffer RB** into the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through. Add **500 µL RNA Wash Buffer** to the column and centrifuge at 14,000 rpm for 1 min. Discard the flow-through. Note: DNase I set can be purchased from Biomiga separately.

7. Add **500 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow through and reuse the collection tube.

8. Centrifuge the empty column, **with the lid open**, at 12,000 rpm for 1 min. Residual ethanol will be removed more effectively with the column lid open.

9. Transfer the column to a RNase-free 1.5 mL tube and add **50-100 µL DEPC-treated water** to the column. Centrifuge at 12,000 rpm for 1 min. The RNA is in the flow-through liquid. Store RNA at -20 °C.

Note: It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. An $A_{260/280}$ ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

Trouble Shooting Guide

Problem	Possible reason	Suggested Improvement
Low $A_{260/280}$ ratios	Protein contamination	Do a Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
Low $A_{260/280}$ ratios	Guanidine Thiocyanate contamination	Add 2.5 volumes of ethanol and 0.1M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at -20 °C. Centrifuge at 10,000 g for 15 min at 4°C. Resuspend the RNA pellet in DEPC-treated water.
Low Yield	RNA in sample degraded	Freeze samples immediately in liquid nitrogen and store at -70 °C after collect it.
Low Yield	The binding capacity of the membrane in the spin column was exceeded	Use of too much tissue sample exceeding the binding capacity of spin column will cause the decreasing of total RNA yield.
Low Yield	Ethanol not added to buffer	Add ethanol to the RNA Wash Buffer and DNase Stop Solution before purification.
Genomic DNA contamination	Too much total RNA sample was used in RT-PCR.	Reduce total RNA amount used in RT-PCR to 50-100 ng.
Genomic DNA contamination	The sample may contain too much genomic DNA.	Reduce the amount of starting tissue in the preparation of the homogenate. Most tissues will not show a genomic DNA contamination problem at 30 mg or less per prep. Reduce cell numbers to 1-2x10^6 or increase buffer volume and do multiple loadings to column.