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EasyRNA™ Cell/Tissue RNA Mini Kit

(Cat# K1337-50, -250; Store at RT)

I. Introduction:

BioVision's EasyRNA™ Cell/Tissue RNA Mini 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. This kit purifies up to 100 µg of total RNA from eukaryotic cells or animal tissues. The purified RNA is ready for RT-PCR, Northern blotting, polyA+RNA purification, nuclease protection, and *in vitro* translation.

II. Sample Type:

For fast and efficient isolation of total RNA from tissues, cultured cells within 30 min.

III. Kit Contents:

Components	K1337-50 50 Preparations	K1337-250 250 Preparations	Part Number
Buffer LY	28 mL	135 mL	K1337-XX-1
Buffer RB	22 mL	120 mL	K1337-XX-2
RNA Wash Buffer*	12 mL	50 mL	K1337-XX-3
DEPC-Treated ddH_2O	10 mL	30 mL	K1337-XX-4
RNA Columns	50	250	K1337-XX-5

DNase I is not supplied. *They could be purchased separately. *Add 48 mL (K1337-50) or 200 mL (K1337-250) 100% ethanol to RNA Wash Buffer before use.*

IV. User Supplied Reagents and Equipment:

- Tabletop microcentrifuge and 1.5 mL sterile tubes
- 100% ethanol
- β-mercaptoethanol
- **Optional:** DNase I

V. Shipment and Storage:

All the reagents are shipped at room temperature. DNase I (optional) should be stored at -20°C. All other components can be stored at room temperature. All kit components are guaranteed for 12 months from the date of purchasing. DO NOT FREEZE!

VI. Reagent Preparation and Storage Conditions:

- Prepare all components and get all necessary materials ready by examining the protocol and be familiar with each step.
- Add 1% volume of β-mercaptoethanol to Buffer LY before use and store at 4°C.
- Add 48 mL (K1337-50) or 200 mL (K1337-250) 100% ethanol to RNA Wash Buffer before use.
- All centrifugation steps should be carried out at room temperature.

VII. 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 g for 5 min. Wash the cell pellet with 4°C PBS and centrifuge at 300 g for 5 min. Discard the supernatant.
b. For total RNA extraction from adherent cultured cells, remove the culture medium. Wash the cell by 4°C PBS and remove the PBS.
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. If the solution is clear, go to step 5, otherwise go to step
4. Centrifuge the solution at top speed for 1 min and transfer the clear lysate to a clean 1.5 mL tube.
5. 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 there is any precipitation.
6. 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.
7. Add 400 µL Buffer RB to the column and centrifuge at 14,000 rpm for 1 min. Discard the flow-through. Ensure that ethanol has been added to RNA Wash Buffer before use.
8. *Optional: Add 50 µL DNase I (5U, 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 14,000 rpm for 1 min. Discard the flow-through. Add 300 µL RNA Wash Buffer to the column and centrifuge at 14,000 rpm for 1 min. Discard the flow-through.* Note: DNase I can be purchased separately.
9. Add 500 µL RNA Wash Buffer to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through. Repeat once.
10. Centrifuge the empty column, with the lid open, at 13,000 rpm for 1 min. *Note: Residual ethanol will be removed more effectively with the column lid open.*
11. Transfer the column to an RNase-free 1.5 mL tube and add 50-100 µL DEPC-treated water to the column. Centrifuge at 14,000 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 A260/A280 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 and transfer the tissue into a 1.5 mL tube containing 500 µL Buffer LY and 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 for 10 min at 14,000 rpm 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 the binding 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 400 µL Buffer RB to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through.
Ensure that ethanol has been added to RNA Wash Buffer before use.
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 300 µL RNA Wash Buffer to the column and centrifuge at 14,000 rpm for 1 min. Discard the flow-through.* ***Note: DNase I can be purchased separately.***
7. Add 500 µL RNA Wash Buffer to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through. Repeat once.
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 14,000 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 A260/280 ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

Determine amounts of samples to be processed:

The yield depends on the tissue and cells to be processed. Table below shows the typical yield of total RNA per column

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 preparations:** (Do not use more than $5x10^6$ cells)

- **Suspension cultured cells:** Determine the cell numbers and collect cells by centrifuging at 300g for 5 min. Remove all supernatant completely by aspiration and proceed to step 2.
- **Adherent cultured cells:** Determine cell numbers and aspirate the medium completely with a Pasteur pipet. Go to step 2 immediately by adding Buffer LY. *Note: Supernatant must be removed completely. Residual supernatant will inhibit cell lysis and thus affect the RNA yield.*

2. **Suspension cells:** Flicking the tube to loosen the cell pellet and add 500 µL Buffer LY.
Adherent cells: Add 500 µL Buffer LY directly into the dish. Use pipet tip to mix and transfer the cell lysate to a 1.5 mL tube.
Note: Determine the volume of Buffer LY to be used and add 20 µL of β- mercaptoethanol (β-ME) per 1 mL Buffer LY before use. Buffer LY contains β- ME can be stored at room temperature for up to 1 month.
Note: β-ME is recommended for RNases rich cell lines.
3. Homogenize the lysate by vortexing vigorously or repeated pipetting until the sample is uniformly homogenized. If the solution is clear, go to step 5, otherwise go to step 4.
4. Centrifuge the solution at 13,000 rpm for 2 min and transfer the clear lysate to a clean 1.5 mL tube.
5. 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.
6. Transfer the solution to a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column into a new collection tube.
7. Add 500 µL Buffer RB to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through.
8. Add 500 µL RNA Wash Buffer (Add ethanol before use) to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through.

9. **Optional:** Add 50 µL DNase I (5U，RNase-free) Mixture onto the middle of the column and incubate at room temperature for 15 min. Add 200 µL DNase Stop Buffer onto the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through. Add 300 µL RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.
10. Add another 600 µL RNA Wash Buffer to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through and collection tube, put the column, with the lid open, into a new collection tube. Centrifuge the column at 12,000 rpm for 1 min. *Note: The residual ethanol will be removed more efficiently with the lid of the column open.*
11. Transfer the column to a RNase-free 1.5 mL tube and add 50-100 µL DEPC-treated ddH_2O to the center of the column. Centrifuge at 13,000 rpm for 1 min to elute the RNA. Store the RNA solution at -20°C.

Protocol for Extracting Total RNA from Animal Tissue:

1. Quickly weight an appropriate mass tissue according to Table 1 (Page 6) and transfer the tissue into a 1.5 ml tube containing 500 µL Buffer LY (add β- mercaptoethanol before use) and homogenize the tissue by a rotor starter or ultrasonic homogenizer on ice. Note: Determine the volume of Buffer LY to be used and add 20 µL of β- mercaptoethanol (β-ME) per 1 mL Buffer LY before use. Buffer LY contains (β-ME) can be stored at room temperature for up to 1 month. *Note: Use of too much mass of tissue per preparation will cause genomic DNA contamination.*
2. Centrifuge the lysate for 5 min at 13,000 rpm at room temperature and transfer the cleared lysate to a clean 1.5 ml tube.
3. Add 0.5 volume of the 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 13,000 rpm for 1 min. Discard the flow-through liquid and collection tube, put the column into a new collection tube.
5. Add 500 µL Buffer RB to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through liquid and put the column back to the collection tube.
6. Add 500 µL RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.
7. Optional: Add 50 µL DNase I (5U，RNase-free) mixture onto the middle of the column and incubate at room temperature for 15 min. Add 200 µL DNase Stop Buffer onto the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through. Add 300 µL RNA Wash Buffer to the column and
8. Add 600 µL RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through and collection tube, put the column, with the lid open, into a new collection tube.
9. Centrifuge the column at 13,000 rpm 2 min. It is critical to remove residue ethanol for optimal elution in the following step.
10. Place the column to a RNase free 1.5 mL tube and add 50-100 µL DEPC treated ddH_2O to the column and centrifuge at 13,000 rpm for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20°C.

VIII. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Low A_{260}/A_{280} ratio	• Protein contamination • Guanidine Thiocyanate contamination	• Do a Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected. • Add 2.5 volumes of ethanol and 0.1M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at –20°C. Centrifuge at 10,000g for 15 min at 4°C. Resuspend the RNA pellet in DEPC-treated water.
Low Yield	• RNA in sample degraded. • The binding capacity of the membrane in the spin column was exceeded • Ethanol not added to buffer	• Freeze samples immediately in liquid nitrogen and store at -70°C after collect it. Prepare fresh culture. • Use of too much tissue sample exceeding the binding capacity of spin column will cause the decreasing of total RNA yield. • 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. • The sample may contain too much genomic DNA.	• Reduce total RNA amount used in RT-PCR to 50-100 ng. • 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 1-2x10^5 or increase buffer volume and do multiple loadings to column.