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EasyRNA™ Blood RNA Mini Kit

(Cat# K1373-50, -250; Store at RT)

I. Introduction:

BioVision's EasyRNA™ Blood RNA Mini Kit combines the reversible binding properties of HiBind RNA technology with a specially designed buffer system which can effectively remove DNA before RNA isolation. Samples are first lysed and homogenized in a specially designed denaturing buffer, which immediately inhibits the activity of RNase. The lysate is then passed through a DNA Clearance column which traps the genomic DNA. The flow-through lysate is then applied to RNA column to bind RNA. After two wash steps, purified RNA is eluted with RNase-free water. The whole protocol can be completed in less than 30 minutes. RNA purified using the DNA/RNA method is ready for applications such as RT-PCR, qPCR, differential display, microarrays, etc.

II. Sample Type: For fast and efficient isolation of total RNA.

III. Kit Contents:

	K1373-50	K1373-250	
Components	**50 Preparations**	**250 Preparations**	**Part Number**
Buffer LY	40 mL	150 mL	K1373-XX-1
Buffer RB	50 mL	150 mL	K1373-XX-2
RNA Wash Buffer*	12 mL	50 mL	K1373-XX-3
DEPC-Treated ddH_2O	10 mL	30 mL	K1373-XX-4
RNA Columns	50	250	K1373-XX-5
Homogenization Column	50	250	K1373-XX-6
10X RBC Buffer	200 mL	1 L	K1373-XX-7

**Add 48 mL (K1373-50) or 200 mL (K1373-250) 100% ethanol to RNA Wash Buffer before use. **Add 9.6 mL (K1373-50) or 48 mL (K1373-250) 100% ethanol to DNase Stop Buffer before use.*

IV. User Supplied Reagents and Equipment:

- Tabletop microcentrifuge
- Sterile RNase free 1.5 mL centrifuge tubes and tips.
- Vacuum manifold if use vacuum protocol
- 100% ethanol
- β-mercaptoethanol or DTT

V. Shipment and Storage:

All the reagents are shipped at room temperature. All 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 become familiar with each step.
- Determine the volume of Buffer HLY to be used and add 20 µL of β-mercaptoethanol (βME) per 1 mL Buffer HLY before use. Buffer HLY/β-ME can be stored at room temperature for up to 1 month. ***Note: 10 mM DTT (final concentration of 10 mM) can be used to replace β-mercaptoethanol.***
- Add **48** mL (K1373-50)/**200** mL (K1373-250) 100% ethanol to RNA Wash Buffer before use. The final ethanol is 80%(v/v).
- RBC Buffer is supplied as 10X concentrate. Calculate the amount of buffer to be used and mix one part of 10X RBC Buffer with 9 parts of ddH_2O before use.

VII. Protocol for Total RNA Extraction from Tissue Samples:

Disruption and homogenization of tissue samples:

It is critical to disrupt and homogenize the samples completely and properly for high quality RNA yield. The purpose for homogenization is to reduce the viscosity by shearing genomic DNA and other high molecular weight cell components to create a homogenous lysate. Incomplete homogenization may result in clogging the column and reducing the RNA yield.

1. Sample disruption by mortar and pestle

A. Excise tissues and freeze in liquid nitrogen immediate.
B. Grind the sample with ceramic mortar and pestle to a fine powder under liquid nitrogen.
C. Transfer the suspension into a tube pre-chilled in liquid nitrogen and allow the liquid nitrogen to evaporate while the samples remain frozen.
D. Add Buffer HLY before the sample gets thawed.

2. Homogenization using homogenization columns

Up to 700 µL of samples can be loaded per column.

3. Rotor-Stator for sample disruption and homogenization

Using a proper size probes and generator, the process simultaneously disrupts and homogenizes most of samples.

4. Bead milling for sample disruption and homogenization

Cells and tissues can be disrupted and homogenized by rapid agitation in the presence of glass beads in Buffer HLY. Use 4-8 mm glass beads for animal tissues, 0.5 mm for yeast cells and 0.1 mm for bacterial samples.

Removal of genomic DNA using DNAse digestion

For most of the downstream applications, DNase digestion is not necessary since the genomic DNA removal column and the RNA column remove nearly all genomic DNA. However, certain sensitive downstream applications may require further DNase I digest.

Stabilization of RNA in harvested animal tissues:

The intact of RNA in harvested tissue will be protected with the addition of RNASecure solution

A. Cut the tissue into slices less than 0.5 cm thick and immediately add at least 15 volumes of RNAsecure solution, for example, 150 µL RNAsecure solution per 10 mg tissue.
B. Store at room temperature for up to 24 hours, at 4°C for up to a week, and -20°C or -80°C for long term.

RNA quality

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.

Determine amounts of samples to be processed:

Samples	Buffer HLY (500 µL)	Buffer HLY(700 µL)
Cell numbers	= <5 x10^6	>5x10^6-1x10^7
Tissue mass	< 15 mg	>16-30 mg

The yield depends on the tissue and cells to be processed. Please reference Table 1 to determine the amount of sample and expected yield.

Table 1. Typical yield of total RNA per column

Sample	10 mg/500 µL Buffer HLY	Total RNA Yield (µg)
Liver	10 mg	50 (10 mg tissue)
Kidney	10 mg	20-30 (10 mg tissue)
Muscle*	10 mg	20 (10 mg tissue)
Spleen	10 mg	30-40 (10 mg tissue)
Heart*	10 mg	50 (10 mg tissue)
Brain**	10 mg	80 (10 mg tissue)
Lung	10 mg	10-20 (10 mg tissue)
Pancreas	10 mg	20 (10 mg tissue)
HeLa Cells	$1x10^6$	15 ($1x10^6$ cells)
293HEK	$1x10^6$	12 ($1x10^6$ cells)
COS-7	$1 x10^6$	30 ($1x10^6$ cells)
NIH/3T3	$1x10^6$	10 ($1x10^6$ cells)

Protocol for extracting total RNA from blood:

1. Add 5 volumes RBC Lysis Buffer in an appropriately sized tube (not provided). Vortex to mix thoroughly. For example, add 5 mL RBC Buffer to 1 mL blood in a 15 mL conical tube or add 500 µL RBC Buffer to 100 µL blood in a 1.5 mL microcentrifuge tube. ***Note:*** *RBC Lysis Buffer is supplied as a 10X concentrate. Dilute with nuclease free ddH2O before use.*
2. Let it sit on ice for 15 minutes. Vortex twice during incubation. *Note: Lysis of red blood cells is indicated when the solution becomes translucent. For blood samples from individuals with an elevated hematocrit or elevated erythrocyte sedimentation rate (ESR), extend the incubation time to 20 minutes.*
3. Centrifuge at 400*g* at 4°C for 10 minutes to pellet leukocytes. Completely remove and discard the supernatant. If a refrigerated centrifuge is not available, centrifuge at room temperature, but quickly complete Step 4 below.
4. Add 2 volumes of of RBC Lysis Buffer per volume of whole blood used in Step 1. Vortex to resuspend cells. *Note: For example, if you began the protocol with 1 mL whole blood, wash the leukocyte pellet with 2 mL RBC Lysis Buffer.*
5. Centrifuge at 400*g* at 4°C for 10 minutes. Completely remove and discard the supernatant. If a refrigerated centrifuge is not available, centrifuge at room temperature, but quickly complete Step 6 below.
6. Add Buffer HLY to the pelleted white blood cells. For <500 µL whole blood, add 400 µL Buffer HLY. For 0.5-1.0 mL whole blood, add 700 µL Buffer HLY. Vortex to mix thoroughly. Samples may be safely stored at -70°C after addition of Buffer HLY.
7. Transfer the cell lysate directly to the Homogenization Column and centrifuge at 10,000 rpm for 2 min.
8. Save the filtrate and discard the Homogenization Column.
9. Add an equal volume of 70% ethanol. Vortex to mix. A precipitate may form after the addition of ethanol. This will not interfere with RNA

isolation.

10. Transfer 700 µL (including any precipitate) to a RNA Mini Column.
11. Centrifuge at >10,000 x g for 30 seconds. Discard the filtrate and reuse the Collection Tube. Process the remaining sample as described if any.
12. Add 500 µL Buffer HRB to the RNA column and centrifuge at >10,000g for 30 seconds. Discard the filtrate and the Collection Tube.
13. Add 700 µL RNA Wash Buffer and centrifuge at >10,000g for 30 seconds. Discard the filtrate and reuse the Collection Tube.
14. Centrifuge the RNA column at maximum speed for 2 min to dry the membrane.
15. Transfer the RNA column to a clean 1.5 mL vial and add 50-100 µL DEPC Water.
16. Centrifuge at >10,000g for 1 min to elute the RNA. Store RNA at -80°C.

Protocol for animal tissue total RNA extraction:

1. Quickly weight an appropriate mass tissue according to Table 1 and immediately transfer the tissue into a 1.5 ml tube containing **500 µL Buffer HLY** (add β-mercaptoethanol before use) and homogenize the tissue by a rotor starter or ultrasonic homogenizer on ice. ***Note:*** *Determine the volume of Buffer HLY to be used and add 20 µL of βmercaptoethanol (βME) per 1 mL Buffer HLY before use. Buffer HLY contains (βME) can be stored at room temperature for up to 1 month.* **Note: Do not use over 30 mg of tissue per column as this causes incomplete tissue digestion and genomic DNA contamination.**
2. Centrifuge the lysate for 2 min at 13,000 rpm at room temperature and transfer the cleared lysate to a DNA Clearance column.
3. Centrifuge at 13,000 rpm for 30s. Discard the DNA Clearance column and save the flow-through.
4. Add 0.5 **volume** of **100% ethanol** into the flow-through **(for example: 250 µL 100% ethanol** for **500 µL flow-through)**, mix thoroughly by pipetting up and down 10 times. Do not centrifuge.
5. Transfer the solution to a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and re-use the collection tube. Process the remaining sample.
6. Add **500 µL Buffer HRB** to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through liquid and re-use the collection tube.
7. Add **600 µL RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.
8. Centrifuge the column at 13,000 rpm 2 min. It is critical to remove residue ethanol for optimal elution in the following step.
9. Place the column to a RNase free 1.5 mL tube and add **50-100 µL** RNA Elution buffer (**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. Optional: Add the eluted RNA back to the column for another elution may yield additional 20-30% of the RNA. The first elution normally yields 70-80% of the RNA.

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 vol 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. • 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.

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