

EXOBX® RNA Isolation Kit (Purification Column-Based)

Cat. no. R6309

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Catalog Number	Reagent In Kit	Size	Storage
R6309	Lysis Buffer	30 mL	RT
	Wash Buffer A	30 mL	RT
	Wash Buffer B	15 mL	RT
	Elution Buffer R	10 mL	RT
	gDNA Clear Column	50 sets	RT
	RNA Purification Column	50 sets	RT
	Manual	1	

1. Transportation and Storage

This product is transported and stored at room temperature, and valid for 2 years.

2. Important Product Information

- 1) This kit could isolate high pure RNA within 20 minutes.
- 2) The whole experimental process should be carried out on ice.
- 3) Repeat freezing and thawing for samples may promote RNA degradation, please use fresh samples or samples stored at -80 °C to isolate RNA.
- 4) Please use RNase-free appliances and consumables, such as microcentrifuge tubes, tips and pipette.
- 5) The absorbance values at 260 nm, 320 nm, 230 nm and 280 nm represent the absorbance values of nucleic acid, background (solution turbidity), salt concentration and protein and protein, respectively. Therefore, the ratio of OD260/OD280 (R) was usually used to judge RNA purity. The values of R for RNA with high purity are usually within the range of 1.8-2.2, especially 2.0-2.2. When R <1.8, the isolated RNA usually contains protein contamination. However, when R >2.2, few RNA may have been degraded into single nucleotide.
- 6) RNA quality may also be determined by agarose gel electrophoresis, high-quality total RNA has the following characteristics: clear electrophoresis band, no tailing or blurring; The brightness of 28 s band should be obviously greater than 18 s band, and the ratio is close to or more than 2:1. The brightness of 5 s band is weak or invisible.
- 7) RNA isolated by this kit contains 28 s and 18 s RNA, but not 5 s RNA, and please use microRNA Isolation Kit (Omn-04) to isolate 5 s RNA, including microRNA and siRNA, etc.
- 8) gDNA Clear Column can effectively remove genomic DNA, and improve the RNA purity.

9) Please wear lab clothes, gloves and safety glasses during the experiment.

3. Product Introduction

The kit could rapidly isolate RNA from cultured cells and animal tissues based on the unique Lysis Buffer and RNA Purification Column. Moreover, gDNA Clear Column efficiently remove genomic DNA from lysate, which facilitate the isolation of high pure RNA. The isolated RNA could be used for various subsequent experiments, including reverse transcription, real-time fluorescence quantitative PCR (QPCR), Northern Blot, sequencing and other experiments.

4. Additional Materials Required

- Ethanol
- 70% ethanol
- Variable-speed bench-top microcentrifuge (refrigerated)
- RNase-free tubes and tips
- Vortex Mixer

5. Experimental Procedure

1) Reagents Preparation. Add 350 mL ethanol to Wash Buffer B and mix thoroughly.

2) Sample preparation.

- a) Cell sample. Pellet cells by centrifuging harvested cell suspension in a 1.5 mL microcentrifuge tube at 1000 g for 3 minutes. Carefully remove and discard the supernatant. Add Lysis Buffer according to Table 1, mix thoroughly and incubate at room temperature for 3-5 minutes.
- b) Tissue sample. Tissues were cut into pieces, suspended with Lysis Buffer according to Table 2, and then homogenized until the mixture is transparent by using Handheld Homogenizer. Incubate at room temperature for 3-5 minutes.

Table 1. Lysis Buffer Volume for Cells	
Cell Number	Lysis Buffer
Less than 5×10 ⁶	350 µl
5-10×10 ⁶	600 µl

Table 2. Lysis Buffer Volume for Tissues	
Tissue weight	Lysis Buffer
Less than 10 mg	350 µl
10-20 mg	600 µl

3) Transfer the lysates to gDNA Clear Column.

4) Centrifuge at 12 000 g for 1 min at 4 °C, discard DNA Clear Column.

5) Add equal volume of 70% ethanol to the filtrate, and mix it upside down.

6) Transfer the full mixture to RNA purification column.

7) Centrifuge at 12, 000 g for 2 min at 4 °C, discard filtrate.

- 8) Add 0.5 ml Wash Buffer A to RNA Purification Column, and centrifuge at 12 000 g for 1 min at 4 °C, discard filtrate.
- 9) Add 0.5 ml Wash Buffer B to RNA Purification Column, and centrifuge at 12 000 g for 2 min at 4 °C, discard filtrate.
- 10) Transfer RNA Purification Column to a new microcentrifuge tube, and add 100 Elution Buffer R (or RNase-free ddH₂O) into RNA Purification Column, incubate at room temperature for 2 min.
- 11) Centrifuge at 12 000 g for 2 min at 4 °C, the filtrate is the isolated RNA solution.
- 12) The isolated RNA may be used to measure concentration immediately or stored at -80 °C.

6. Identification of Isolated RNA

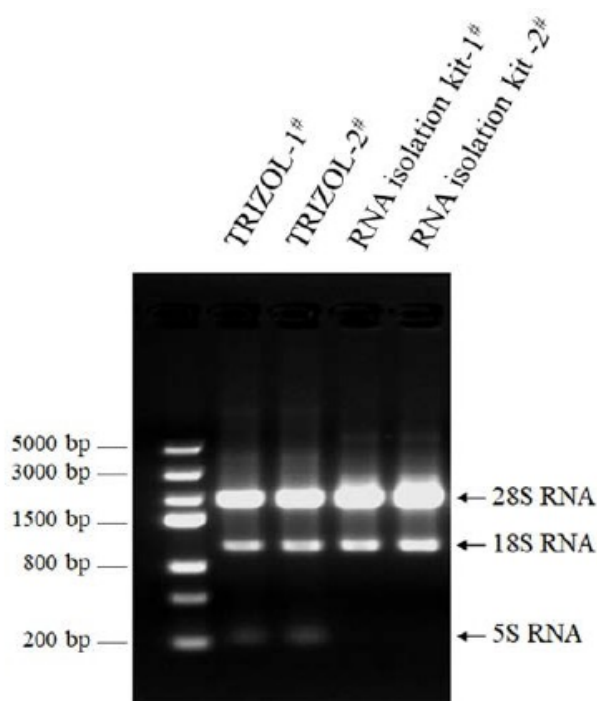


Figure 1. Identification of RNA isolated from HepG2 cells by agarose gel electrophoresis.

7. Frequently Asked Questions and Analysis

Questions	Reasons	Solutions
The isolated RNA has been degraded	The samples have been frozen and thawed repeatedly.	Use fresh samples to isolate RNA.
	The samples have not been stored below -80 °C.	Store samples at -80 °C or in the liquid nitrogen
	The reagents and materials used in the experiment are not RNase-free.	Use RNase-free reagents, tubs and tips.
	The RNA isolation process does not carry out on ice.	Isolate RNA from samples on ice.
	The elution buffer for RNA is not RNase-free.	Use RNase-free elution buffer or ddH ₂ O to elute RNA pellet.
	The storage of RNA is improper.	The isolated RNA should be stored at -80 °C, but not -20 °C.
The concentration of isolated RNA is low.	Less samples were used in RNA isolation.	Increase samples for RNA isolation.
	Samples were not lysed completely.	Increase Lysis Buffer, or prolong the time for samples lysing.
	Less Elution Buffer R (<50 µl) was used to elute RNA from RNA Purification Column.	Increase Elution Buffer R to elute RNA.
	The Elution Buffer R is not dripped onto the membrane of RNA Purification Column.	Drip Elution Buffer R on the membrane of RNA Purification Column.
	The incubation time for Elution Buffer R and membrane of RNA Purification Column is short.	Increase the incubation time to 5 minutes.
The isolated RNA has been contaminated by genomic DNA.	Excessive samples were used for RNA isolation.	Reduce samples in RNA isolation.