

EXOBX® RNA Isolation Reagent (TRIZOL)

Cat. no. R6308

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Catalog Number	Reagent In Kit	Size	Storage
R6308	RNA Isolation Reagent(TRIZOL)	100 mL	4 °C
	RNA dissolving solution	10 mL	4 °C
	Manual	1	

1. Transportation and Storage

This product is transported at room temperature, stored at 4 °C and valid for 2 years.

2. Important Product Information

- 1) If samples have a high content of fat, protein and polysaccharide, centrifuge the lysate for 5 minutes at 12,000 g at 4 °C, then transfer the clear supernatant to a new tube.
- 2) If the samples are small, please use RNA Purification Mini Kit (Magnetic Beads-Based) (Omn-12) to isolate RNA.
- 3) The whole experimental process should be carried out on ice.
- 4) Repeat freezing and thawing for samples may promote RNA degradation, please use fresh samples or samples stored at -80 °C to isolate RNA.
- 5) Please use RNase-free appliances and consumables, such as microcentrifuge tubes, tips and pipette.
- 6) 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.
- 7) 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.

8) Please wear lab clothes, gloves and safety glasses to avoid chemical burns of skin, eyes or respiratory tract caused by reagent splash. In case of contact with skin or eyes, please immediately rinse with plenty of water and go to the hospital for care.

3. Product Introduction

The EXOBX®™ RNA Isolation Reagent (TRIZOL) enables isolation of total RNA from various samples, including cell, bacteria, blood, animal and plant tissues. The isolated total 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

- 75% Ethanol
- Isopropyl alcohol
- Chloroform
- Variable-speed bench-top microcentrifuge (refrigerated)
- RNase-free tubes and tips.
- Vortex Mixer

5. Experimental Procedure

1) Sample preparation.

a) Cell sample. Pellet 1×10^6 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 1 ml RNA Isolation Reagents (TRIZOL), mix thoroughly and incubate at room temperature for 3-5 minutes.

b) Tissue sample. Tissues (more than 10 mg) were cut into pieces, suspended with 1 ml RNA Isolation Reagents (TRIZOL), and then homogenized until the mixture is transparent by using Handheld Homogenizer. Incubate at room temperature for 3-5 minutes.

2) Add 0.2 mL of chloroform per 1 mL of RNA Isolation Reagent (TRIZOL) used for lysis, securely cap the tube, then thoroughly mix by vortexing.

3) Incubate for 2-3 minutes.

4) Centrifuge the sample for 15 minutes at 12 000 g at 4 °C.

5) The mixture separates into a lower red phenol-chloroform, an interphase, and a colorless upper aqueous phase.

6) Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out.

NOTE: Avoid transferring any of the interphase or organic layer into the pipette when removing the aqueous phase.

7) Add 0.5 mL of isopropanol to the aqueous phase, per 1 mL of RNA Isolation Reagent (TRIZOL) used for lysis.

8) Incubate for 10 minutes at 4 °C, centrifuge at 12 000 g for 10 minutes at 4°C, the total RNA precipitate forms a white gel-like pellet at the bottom of the tube.

9) Discard the supernatant with a micropipette.

10) Centrifuge at 3000 g for 1 min, and remove the residual liquid with a pipette.

11) Add 100 µL RNA Dissolving solution (or RNase-free ddH₂O), mix thoroughly to get RNA solution.

12) The isolated RNA may be used to measure concentration immediately or stored at -80 °C.

6. Identification of Isolated RNA

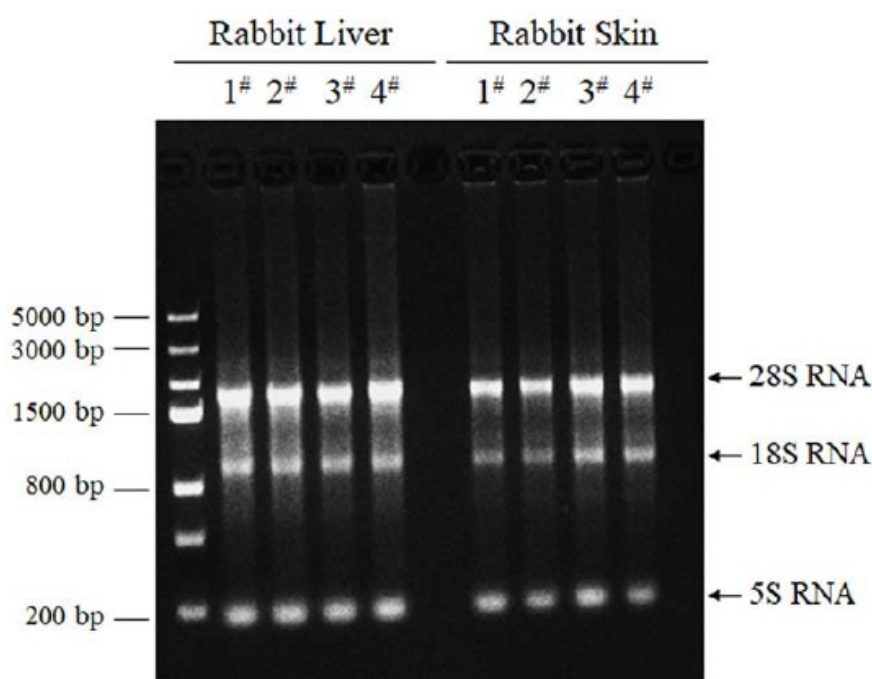


Figure 1. Identification of RNA isolated from rabbit tissues by agarose gel electrophoresis.

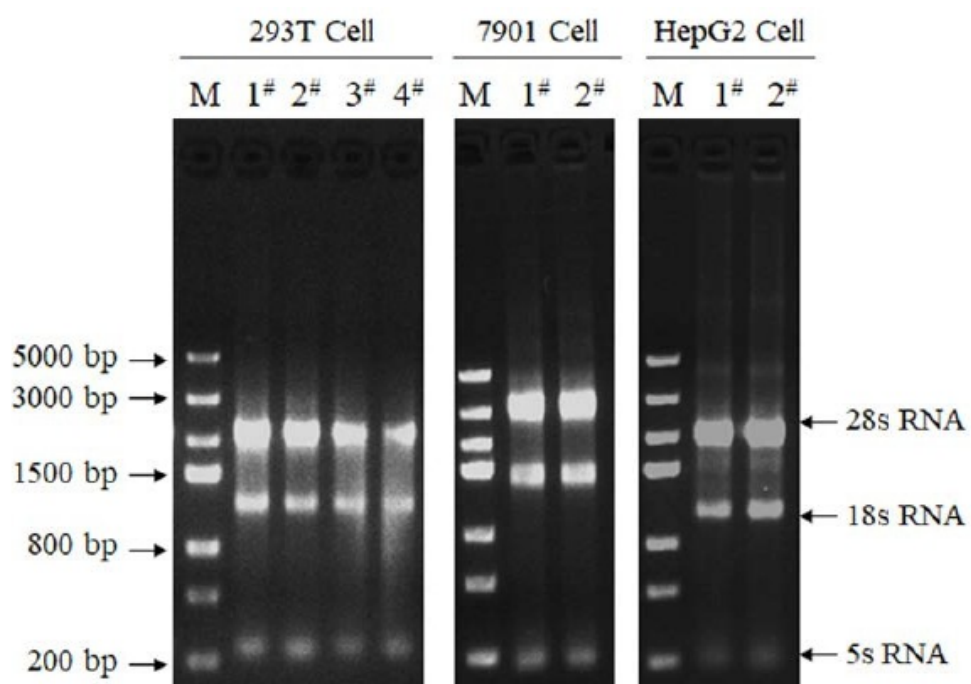


Figure 2. Identification of isolated RNA from different kinds cells by agarose gel electrophoresis.

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 dissolving buffer for RNA is not RNase-free.	Use RNase-free dissolving buffer or ddH ₂ O to dissolve 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 by RNA Isolation Buffer completely.	Increase the volume of RNA Isolation Reagent, or prolong the time for samples lysing.
The isolated RNA has been contaminated by genomic DNA.	Excessive samples were used for RNA isolation.	Reduce samples in RNA isolation.
	Transfer any of the interphase or organic layer into the pipette when removing the aqueous phase.	Avoid the error operation during the experiment.