

EXOBX® RNA Purification Mini Kit (Magnetic Beads-Based)

Cat. no. R6311

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Product Information

Product	EXOBOX® RNA Purification Mini Kit (Magnetic Beads-Based)
Catalog No.	R6311
Storage conditions	Transported at room temperature; store at 4 °C. Shelf life 2 years.
Applicable samples	Cells ($< 1 \times 10^5$) and tissues (< 10 mg)

Kit Contents

Reagent in Kit	Size	Storage
RNA Isolation Reagent (TRIZOL)	50 mL	4 °C
Magnetic Beads	2.5 mL	4 °C
Elution Buffer R	10 mL	4 °C
Manual	1	—

Transportation and Storage

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

Product Introduction

The kit is specifically designed for isolating total RNA from trace samples, such as cells ($< 1 \times 10^5$) and tissues (< 10 mg), based on an RNA isolation reagent (TRIZOL) and magnetic beads. The isolated total RNA can be used for various downstream applications, including reverse transcription, real-time fluorescence quantitative PCR (qPCR), Northern blot, sequencing and other experiments.

Additional Materials Required

- 75% Ethanol
- Isopropyl alcohol
- Chloroform
- Variable-speed bench-top microcentrifuge (refrigerated)
- RNase-free tubes and tips
- Vortex mixer
- Magnetic rack
- Rotary mixer

Experimental Procedure

1. Sample preparation.

- 1. Cell sample.** Pellet $1-5 \times 10^5$ cells by centrifuging harvested cell suspension in a 1.5 mL microcentrifuge tube at $1000 \times g$ for 3 minutes. Carefully remove and discard the supernatant. Add 1 mL RNA Isolation Reagent (TRIZOL), mix thoroughly and incubate at room temperature for 3-5 minutes.

2. **Tissue sample.** Cut tissues (10 mg) into pieces, suspend with 1 mL RNA Isolation Reagent (TRIZOL), and homogenize until the mixture is transparent using a 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 $12,000 \times g$ at 4°C .
5. The mixture separates into a lower red phenol-chloroform phase, 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. Add 50 μL magnetic beads (vortex the magnetic beads before use), mix thoroughly, and incubate for 30 minutes at 4°C using a rotary mixer.
9. Pellet the magnetic beads by centrifugation at $3000 \times g$ for 5 minutes, or using the magnetic rack. Carefully remove and discard the supernatant.
10. Add 1 mL 75% ethanol, vortex at medium speed for 30 seconds to completely disperse the magnetic beads.
11. Pellet the magnetic beads by centrifugation or using the magnetic rack. Carefully remove and discard the supernatant.
12. Centrifuge at $3000 \times g$ for 1 minute, and remove the residual liquid with a pipette.
13. Add 100 μL Elution Buffer R (or RNase-free ddH_2O), and vortex for 30 seconds.
14. Centrifuge at $7000 \times g$ for 2 minutes, then place the centrifuge tube on a magnetic rack and transfer the supernatant to a new tube — this is the RNA solution.
15. The isolated RNA may be used to measure concentration immediately or stored at -80°C .

Identification of Isolated RNA

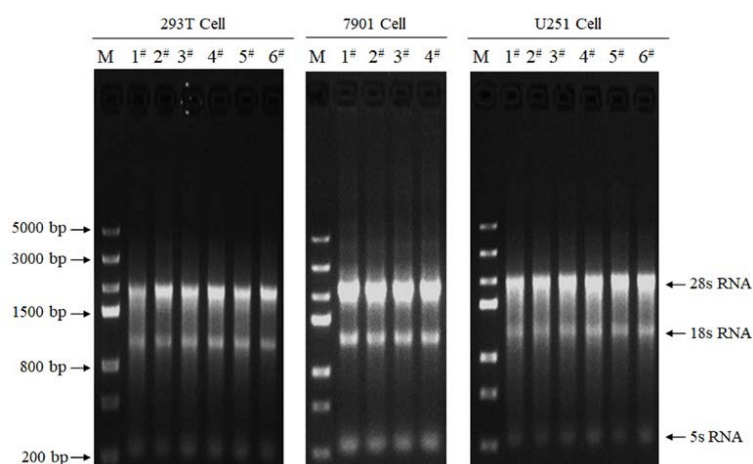


Figure 1. Identification of isolated RNA by agarose gel electrophoresis.

Important Product Information

1. The magnetic beads should be stored at 4°C and must not be frozen, or they become invalid.
2. The whole experimental process should be carried out on ice.

3. Repeated freezing and thawing of 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 pipettes.
5. The absorbance values at 260 nm, 320 nm, 230 nm and 280 nm represent the absorbance of nucleic acid, background (solution turbidity), salt concentration and protein, respectively. The ratio of OD260/OD280 (R) is usually used to judge RNA purity. Values of R for high-purity RNA are usually within 1.8–2.2, especially 2.0–2.2. When $R < 1.8$, the isolated RNA usually contains protein contamination; when $R > 2.2$, some RNA may have degraded into single nucleotides.
6. RNA quality may also be determined by agarose gel electrophoresis. High-quality total RNA shows a clear electrophoresis band with no tailing or blurring; the brightness of the 28S band should be clearly greater than the 18S band, with a ratio close to or above 2:1, while the 5S band is weak or invisible.
7. Please wear lab clothing, gloves and safety glasses to avoid chemical burns to skin, eyes or the respiratory tract caused by reagent splashes. In case of contact with skin or eyes, rinse immediately with plenty of water and seek medical care.

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 liquid nitrogen.
	The reagents and materials used in the experiment are not RNase-free.	Use RNase-free reagents, tubes and tips.
	The RNA isolation process was not carried 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 from magnetic beads.
	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	Fewer samples were used in RNA isolation.	Increase the amount of sample for RNA isolation.
	The concentration of samples is low.	Use samples with higher concentration to isolate RNA.
	The intensity of elution is not enough to elute RNA.	Increase vortex intensity and prolong vortex time.
	The magnetic beads have become invalid and cannot absorb RNA.	Use new magnetic beads.
	More elution buffer was used to elute RNA.	Reduce the elution buffer used in the experiment.