General RNA Extraction Kit

Cat# NB-03-0258

Kit Content

Component	NB-03-0258
	50 preps
Solution RL	60 ml
Wash Buffer RPI	18 ml
Wash Buffer RW	12 ml
DEPC-treated water	10 ml
RNase-free Spin Column	50 each
RNase-free Microcentrifuge Tube	50 each

Materials supplied by the users:

- Chloroform
- Ethanol (96–100%)

Description

General RNA Extraction Kit provides a simple method of isolating total RNA from a wide range of sample types and amounts. In general, samples are lysed and then homogenized in the presence of guanidinium isothiocyanate, a chaotropic salt which is capable of protecting the RNA from endogenous RNases. After homogenization, ethanol is added to the sample. The sample is then processed through a spin column containing a clear silica-based membrane where the RNA binds. Any impurities are effectively removed by subsequent washing. The purified total RNA is then eluted in RNase-Free Water and is suitable for use in a variety of downstream applications.

Downstream Applications

Purified RNA is free of impurities and enzyme inhibitors, and have an A260/280=1.8-2.0, is suitable for applications such as:

- Reverse transcription PCR (RT-PCR)
- Real-time quantitative PCR (qPCR)
- Northern blotting
- Nuclease protection assays
- RNA amplification for microarray analysis
- cDNA library preparation after poly(A)+ selection

Feature

- Stable yield
- Reliable performance of high-quality purified total RNA in downstream applications

Storage

DEPC-treated water store at -20 ˚C, other reagents store at 2-8 ˚C, protect from light. Kit contents are stable for up to 12 months when stored properly.

Important Notes

•Prior to the initial use of the kit, dilute the Wash Buffer RPI and RW with ethanol (96-100%):

Solution	Wash Buffer RPI	Wash Buffer RW
Initial Volume	18 ml	12 ml
Ethanol	12 ml	48 ml
Total Volume	30 ml	60 ml

Mix well, mark the labels on the bottle that ethanol is added.

Precautions for Preventing RNase Contamination

RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA.

• Ensure that no RNases are introduced into the sterile solutions of the kit.
• Only use sterile, disposable RNase-free pipet tips and microcentrifuge tubes.
• Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin. Change gloves frequently, particularly as the protocol progresses from crude extracts to more purified material.
• Always use proper microbiological aseptic techniques when working with RNA.

Recommended volume of Solution RL

10 cm^2 adherent cells	1 ml
10^7 suspension cells	1-2 ml
100 ul white cells	2 ml
50-100 mg ordinary tissue	1 ml
50-100 mg special tissue (liver, spleen, bone or cartilage)	2 ml
15-100 mg plant tissue	1 ml

Protocol

1. Sample processing

• Tissues

Tissue from animal or plant (either fresh or frozen at -70 ˚C until use) can be processed by freezing with liquid nitrogen and grinding into powder using mortar and pestle. Homogenize tissue samples in 1 ml Solution RL per 50-100 mg tissue. Tissue homogenizer or rotor-stator mixer also can be used.

• Adherent Cells
Lyse cells directly in a culture dish by adding 1 ml of Solution RL to the dish and passing the cell lysate several times through a pipet tip. The amount of Solution RL required is based on the culture dish area (1 ml per 10 cm2) but not on the number of cells present.
• Suspension Cells
Harvest cells and pellet cells by centrifugation. Use 1 ml of Solution RL per 5–10×10^6 animal, plant, or yeast cells, or per 1×10^7 bacterial cells. Lyse cells by repetitive pipetting up and down. Do not wash cells before addition of Solution RL to avoid any mRNA degradation. Disruption of some yeast and bacterial cells may require a homogenizer.

2. Incubate at room temperature (15-30 ˚C) for 5 min.

3. Optional • Centrifuge at 12,000 rpm for 5 min at 4 ˚C, transfer the supernatant to a new RNase-free microcentrifuge tube. This step can eliminate protein, lipid, polysaccharide, musle or plant fibre.

4. Add 200 µl (1/5 volume of Solution RL) chloroform, mix by vortexing for 15 sec, incubate at room temperature for 3 min.

5. Centrifuge the sample at 12,000 rpm for 10 min at 4 ˚C. Transfer the colorless upper phase to a new RNase–free tube.
• After centrifugation, the mixture separates into 3 layers: yellow organic phase, an interphase, and a colorless upper aqueous phase which contains the RNA.

6. Add an 0.5× volume of ethanol (96-100%), mix well. Visible precipitate may form after adding ethanol. Transfer the mixture to RNase-free spin column, centrifuge at 12,000 rpm for 30 sec at 4 ˚C, discard the flow-through.

7. Add 500 µl Wash Buffer RPI (check whether ethanol is added or not), Centrifuge at 12,000 rpm for 30 sec at 4 ˚C, discard the flow-through.

8. Add 500 µl Wash Buffer RW (check whether ethanol is added or not), incubate at room temperature for 1 min. Centrifuge at 12,000 rpm for 30 sec at 4 ˚C, discard the flow-through. Repeat this step.

9. Centrifuge at 12,000 rpm for 2 min to eliminate the residual ethanol.
Optional • Open the column to air dry for a few minutes.

10. Place the spin column in a RNase-free microcentrifuge tube, and pipet 30-100 µl DEPC-treated water directly onto the membrane. Incubate at room temperature for 2 min, and then centrifuge at 12,000 rpm for 2 min to elute. The tube contains the purified RNA. Store RNA at -70 ˚C.