General RNA Extraction Kit

Catalog No: R1011 (50 preps) R1012 (200 preps)

Kit Content

Content	R1011	R1012
Solution RL	60 ml	240 ml
Wash buffer RPI	18 ml	72 ml
Wash buffer RW	12 ml	48 ml
DEPC-treated water	10 ml	40 ml
Rnase-free spin column	50 each	200 each
Rnase-free microcentrifuge tube	50 each	200 each

Materials be supplied by the users

·Chloroform
·Ethanol (96–100%)

Description

This 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 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 to which 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.

Applications

Real-time-PCR (RT-PCR)
Real-time quantitative
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

Store

·Store at 2-8°C, protect from light. Kit contents are stable for up to 12 months, when properly stored.

Note

·Wash Buffer RPI and Wash Buffer RW are supplied as a concentrate. Before using for the first time, add ethanol (96–100%, purity grade p.a.) as indicated on the bottle to obtain a working solution.

- Use sterile, disposable, and individually wrapped plastic–ware.
- Use only 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(live, spleen, bone or cartilage)	2 ml
15-30 mg plant tissue	1 ml

Protocol

1. Sample process

Tissues

Tissue from animal or plant (either fresh or frozen at -70°C until use) can be processed using a mortar and pestle. *The tissue sample can be grinded in liquid nitrogen*. Homogenize tissue samples in 1 ml Solution RL per 50–100 mg tissue using a tissue homogenizer or rotor-stator.

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 cm^2) and not on the number of cells present.

Suspension Cells

Harvest cells and pellet cells by centrifugation. Use 1 ml of the 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 TRIzol Reagent to avoid any mRNA degradation. Disruption of some yeast and bacterial cells may require a homogenizer.

2. Incubate at 15-30°C for 5 min, to lyse the nucleiprotein complex completely

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, fat, polysaccharide, musle or plant fibre.
4. Add 200 µl chloroform, mix by vortexing for 15 seconds, incubate at room temperature for 3 min.

5. Centrifuge the sample at 12,000 rpm for 10 minutes at 4°C. **Note:** After centrifugation, the mixture separates into a lower, yellow phenol–chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. Transfer of the colorless, upper phase containing the RNA to a new RNase–free tube.

6. Add an 0.5 volume of ethanol. Mix well, a visible precipitate may form after adding ethanol. Transfer the mixture to a spin column, centrifuge at 12,000 rpm for 30 seconds at 4°C, discard the flow-through.

7. Add 500 µl Wah Buffer RPI (check whether ethanol is added or not), Centrifuge at 12,000 rpm for 30 seconds 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 seconds at 4°C, discard the flow-through. Repeat this step again.

9. Centrifuge the column at 12,000 rpm for 2 min. air dry the column.

10. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl Rnase-free 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 the DNA at -70°C.