General RNA Extraction kit

Cat No: DR1051 Volume: 50 preps

For research use only

Kit Content

Content	DR1051
Solution DRL	32 ml
Wash buffer DRW1	28 ml
Wash buffer DRW2	40 ml
DRE solution	10 ml
Rnase-free spin column	50 each
Rnase-free microcentrifuge tube	50 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. 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℃, protect from light. Kit contents are stable for up to 12 months, when properly stored.

Note

- **Wash Buffer DRW1 and Wash Buffer DRW2 are supplied as a concentrate.**
- **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 DRL

10 cm2 adherent cells	600 µl
107 suspension cells	600 µl
100 ul white cells	600 µl
50-100 mg ordinary tissue	600 µl
50-100 mg special tissue (live, spleen, bone or cartilage)	600 µl
15-100 mg plant tissue	600 µl

Protocol

1. Sample process

Tissues

Tissue from animal or plant (either fresh or frozen at -70 ℃ until use) can be processed by freezing with liquid nitrogen and grinding into a fine powder using a mortar and pestle. Homogenize tissue samples in 1 ml Solution DRLper 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 DRL to the dish and passing the cell lysate several times through a pipet tip. The amount of Solution DRLrequired is based on the culture dish area (1 ml per 10 cm2) and not on the number of cells present.

Suspension Cells

Harvest cells and pellet cells by centrifugation. Use 1 ml of the Solution DRL per 5–10 × 106 animal, plant, or yeast cells, or per 1 × 107 bacterial cells. Lyse cells by repetitive pipetting up and down. Do not wash cells before addition of Deltazol 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 a 1 ml volume of 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 DRW1
Centrifuge at 12,000 rpm for 30 seconds at 4°C, discard the flow-through.

8. Add 500 µl Wash Buffer DRW2, incubate at room temperature for 1 min, Centrifuge at 12,000 rpm for 30 seconds at 4°C, discardthe 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℃。

شرکت ویژن پارس دلتا
شماره های تماس: 02155433092
02164112505
02164112508
Email: v.gene.pars@gmil.com
Website: www.vgenepars.ir