Super RNA extraction Kit for Tissue & cells

Cat No : YT9080 **Size: 50 preps** <<for research use only>>

Kit Contents	50 preps
Solution RL	60 ml
Wash buffer 1	30 ml
Wash buffer 2 (concentrate)	15 ml
Rnase free water	6 ml
FARB mini column	50 each
Elution tube	50 each
Collection tube	100 each

Note: Preparation of wash buffer 2 by adding 60 ml ethanol (96-100)

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

Materials be supplied by the users : Chloroform , Ethanol (96–100%)

Description :This Special 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.

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

Note
- 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

$10cm^2$ 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)	2ml
15-100 mg plant tissue	1 ml

Protocol

1. Sample process

Tissues 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 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 cm2) 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 Solution RL 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.

7.Place a FARB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FARB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the FARB Mini Column back to the Collection Tube.

8. Add 500 µl of Wash Buffer 1 to the FARB Mini Column, centrifugeat at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.

9. Add 750 µl of Wash Buffer 2 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube. -- Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.

10. Repeat step 9 for one more washing.

11. Centrifuge the FARB Mini Column at full speed for an additional 3 min to dry the FARB Mini Column. -- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

12. Place the FARB Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).

13. Add 40 ~ 100 µl of RNase-free ddH2O to the membrane center of the FARB Mini Column. Stand the FARB Mini Column for 1 min. -- Important Step! For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely. -- Important : Do not elute the RNA using RNase-free water less than suggested volume (< 40 µl). It will lower the RNA yield.

14. Centrifuge the FARB Mini Column at full speed for 1 min to elute RNA.

15. Store RNA at -70C.