TIANGEN®

RNAsimple Total RNA Kit

For purification of high pure total RNA

This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetics.

RNAsimple Total RNA Kit

(Spin Column)

Cat. no. GDP419

Kit Contents

	Contents	GDP419 (50 preps)
GDP419H	Buffer RD	12 ml
	Buffer RW	12 ml
	RNase-Free ddH_2O	15 ml
	RNase-Free Columns CR3 set	50
	RNase-Free Centrifuge Tubes (1.5 ml)	50
	Handbook	1
GRK145	Buffer RZ	60 ml

Note: GDP419H, GRK145 are shipped and packaged separatly.

Storage

Buffer RZ should be stored protected from light at 2-8°C for 15 months; others stored at room temperature (15-30°C) for 15 months.

Introduction

RNAsimple Total RNA Kit is a new generation product developed by improvement, which improves the cracking capacity of the pyrolysis solution and the sensitivity of extraction. Meanwhile, the improvement of the silicon matrix membrane enhances the ability of absorbing RNA, resulting in better purity and higher quality of RNA. The kit can rapidly extract total RNA from a variety of cells or tissues, each adsorption column can handle 50-100 mg of tissue or 5×10^6 cells at a time, and can also process a large number of different samples at the same time. The operation was completed within an hour. The extracted RNA is free of DNA and protein contamination and can be used in Northern Blot, Dot Blot, PolyA Screening, *in vitro* transcript, RNase Protecting the Analysis, and Molecular Cloning.

Important Note

For isolation of bacterial RNA, RNAprep Pure Cell/Bacteria Kit should be used (Cat.no. GDP430).

Notes of preventing RNase contamination

1. Change gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-Free plastic and tips to avoid cross contamination.
3. RNA can be protected in Buffer RZ. But RNA must be stored or processed in RNase-Free plastic or glassware. To wipe off RNase, the glassware can be roasted at 150°C for 4 hours, while plastic can be dipped in 0.5 M NaOH for 10 min, washed by RNase-Free ddH_2O thoroughly, and sterilized.
4. Use RNase-Free ddH_2O to prepare solution. (Add DEPC into water in clean glass container to a final concentration of 0.1% (v/v). Incubate overnight and autoclave for 15 min to remove any trace of DEPC.)

Protocol

Before use, add ethanol (96–100%) to Buffer RD and Buffer RW as indicated on the bottle.

1. Preparing samples.
   a. Tissues: Add liquid nitrogen in tissues and grind them thoroughly. Add 1 ml Buffer RZ for per 30–50 mg sample using a power homogenizer. Usually, the volume of tissue sample should not exceed 10% of the volume of Buffer RZ.
   b. Adherent Cells : Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel. Add 1 ml Buffer RZ directly to the cells in the culture dish per 10 cm^2 of culture dish surface area. Pipette the lysate up and down several times until the solution becomes clear. **Note: the volume of Buffer RZ should be determined according to the surface area instead of the number of cells. An insufficient volume can result in DNA contamination of isolated RNA.**
   c. Suspension Cells: Harvest cells by centrifugation and remove culture medium. Add 1 ml of Buffer RZ per 5-10 × 10^6cells from animal or plant. Do not wash cells before addition of Buffer RZ to avoid increased chance of mRNA degradation.
   d. Blood: Take fresh blood, and add three volumes of Buffer RZ. Mix thoroughly. (Recommended amount: 0.75 ml Buffer RZ for 0.25 ml whole blood)
2. Incubate homogenized samples at 15-30°C for 5 min, to permit complete dissociation of the nucleoprotein complex.
3. *Optional step: Centrifuge the sample at 12,000 rpm (~13,400 × g) for 10 min at 4°C. Transfer the supernatant to a fresh micro-centrifuge tube.*

   Note: When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional centrifugation may be required to remove insoluble material from the samples. RNA remains in the upper aqueous phase after centrifugation.
4. Add 200 µl of chloroform . Cap the tube securely and vortex for 15 sec. Incubate for 3 min at room temperature.

   Note: If vortexing is not applicable, shake tube vigorously by hand for 2 min.
5. Centrifuge the sample for 10 min at 12,000 rpm (~13,400 × g) at 4°C. The mixture separates into a lower yellow phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively

in the aqueous phase. Pipette the aqueous phase out into a new tube.

6. Add the 0.5 volume ethanol (96%-100%) to the aqueous phase slowly. Mix thoroughly (precipitate may appear in this step). Transfer the sample, including any precipitate that may have formed, to an RNase-Free Column CR3 placed in a 2 ml RNase-Free Collection Tube. Close the lid gently, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec at 4°C. Discard the flow-through.

   Note: If the sample is more than 700 µl, transfer the sample to CR3 in two times and centrifuge separately.

7. Add 500 µl Buffer RD **(Ensure ethanol has been added)** to the RNase-Free Column CR3. Close the lid gently, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec at 4°C. Discard the flow-through.

8. Add 700 µl Buffer RW **(Ensure ethanol has been added)** to the RNase-Free Column CR3. Close the lid gently, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec at 4°C. Discard the flow-through.

9. Repeat step 8.

10. Set the RNase-Free Column CR3 back to the Collection Tube. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min at 4°C to dry the spin column membrane.

    Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

11. Place the RNase-Free Column CR3 in a new 1.5 ml RNase-Free Collection Tube. Add 30-100 µl RNase-Free ddH_2O directly to the spin column membrane. Close the lid gently, incubate at room temperature (15–30°C) for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min at 4°C to elute the RNA.

    Note: The volume of elution buffer should not be less than 30 µl, or it may affect recovery efficiency. To obtain higher productivity, add the solution gained from step 11 to the center of membrane again, let the columns stand for 1 min, and then centrifuge.

    Purified RNA should be stored at –70°C.