RNAsimple Total RNA Kit

For purification of high pure total RNA

TIANGEN®

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RNAsimple Total RNA Kit

Cat. no. DP419

Kit Contents

Contents	DP419 50 preps
Buffer RZ	60 ml
Buffer RD	12 ml
Buffer RW	15 ml
RNase-Free ddH_2O	15 ml
RNase-Free Spin Columns CR3	50
RNase-Free Collection Tubes 1.5 ml	50
RNase-Free Collection Tubes 2 ml	50
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Storage

Buffer RZ should be stored protected from light at 2-8°C; others stored at room temperature (15-25°C).

Introduction

RNAsimple Total RNA Kit uses a new RNA isolation technology based on Guanidine Thiocyanate / Phenyl method. It contains a unique buffer RZ that minimizes the contamination of genomic DNA and protein. RNAsimple Total RNA Kit can efficiently isolates high pure RNA from blood, cells, tissues and plant samples in one hour. The purified RNA is ready-to-use in downstream applications such as: RT-PCR and real-time RT-PCR, gene-chips assay, northern blot, dot blot, polyA screening, in vitro transcript, and molecular cloning.

Important Note

For isolation of bacterial RNA, RNAprep Pure Kit (For Cell/Bacteria) should be used (Cat.no. DP430).

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 TRNzol. But RNA must be stored or processed in RNase-Free plastic or glassware. To wipe off RNase, the glassware can be roasted at 150℃ for 4 hours, while plastic can be dipped in 0.5 M NaOH for 10min, washed by RNase-Free ddH_2O thoroughly, and sterilized.
4. Use RNase-Free ddH_2O to confect 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

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

1. Homogenizing samples.
   a. Plant (take leaves as an example): Place fresh leaves in liquid nitrogen and grind thoroughly with a mortar and pestle, or grind in Buffer RZ after cut leaves into pieces. This process is suggested to be finished within one minute. Use 1 ml Buffer RZ per 100 mg leaves.
   b. Tissues (take rat liver as an example): Add 1 ml Buffer RZ for per 30–50 mg of liver sample. Homogenize sample

using a power homogenizer. Usually, the volume of tissue sample should not exceed 10% of the volume of Buffer RZ.

c. Adherent Cells (do not use more than 1×10^7 cells): Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. (Cells grown in a monolayer in cell-culture flasks should always be trypsinized.)

   1) Method A: To lyse cells directly. 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. **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.**

   2) Method B: To trypsinize and collect cells. Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.10–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-Free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x *g* for 5 min. Completely aspirate the supernatant.

      Note: Make sure that the supernatant has been completely removed. Residual medium could lead to incomplete lysis of cells and reduced yield of RNA.

d. Suspension Cells: Harvest cells by centrifugation and remove culture medium. Add 1 ml of Buffer RZ per 5×10^6-10^7 cells from animal, plant or yeast, or 1×10^7 cells of bacterial. Do not wash cells before addition of Buffer RZ to

avoid increased chance of mRNA degradation. Samples from some yeast and bacteria maybe need to be homogenized by using a power homogenizer.

e. Blood: Take fresh blood, and add three volumes of Buffer RZ. Mix thoroughly. (Recommended amount: 0.75 ml Buffer RZ for 0.25 whole blood)

2. Incubate homogenized samples at 15-30℃ 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 minutes 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. However, when dealing with fat tissue, the upper phase is a lipid layer that should be discarded. Retain the clean homogenizing part for next step.

4. Add 200 µl of chloroform per 1 ml Buffer RZ used for homogenization. Cap the tube securely and vortex for 15 s. Incubate for 3 minutes 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. Mix thoroughly (participate may appear in this step). Transfer

the sample, including any precipitate that may have formed, to an RNase-Free Spin 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 s 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 to the RNase-Free Spin Column CR3 (Ensure ethanol has been added). Close the lid gently, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s at 4°C. Discard the flow-through.
8. Add 700 µl Buffer RW to the RNase-Free Spin Column CR3 (Ensure ethanol has been added). Close the lid gently, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s at 4°C. Discard the flow-through.
9. Add 500 µl Buffer RW to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s at 4°C. Discard the flow-through.
10. Set the RNase-Free Spin 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 Spin Column CR3 in a new 1.5 ml RNase-Free Collection Tube (supplied). Add 30-100 µl RNase-Free ddH_2O directly to the spin column membrane. Close the lid gently, incubate at room temperature (15–25°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.

Ordering Information

RNA Isolation

Product	Size	Cat. no.
RNAstore Reagent	20 ml	DP408-01
	100 ml	DP408-02

Reverse Transcription

Product	Size	Cat. no.
Quantscript RT Kit	20 µl × 25 rxn	KR103-03
	20 µl × 100 rxn	KR103-04

Real–Time PCR

Product	Size	Cat. no.
SuperReal PreMix (SYBR Green)	50 µl × 50 rxn	FP204-01
	50 µl × 200 rxn	FP204-02
Quant One Step qRT-PCR (SYBR Green) Kit	50 µl × 50 rxn	FP303-01