EasyPrep Total RNA Kit

For purification of total RNA from blood, cells, tissues, and plant samples

Contents

Introduction

The EasyPrep Total RNA Kit provides a new RNA isolation technology based on guanidine thiocyanate or phenyl method. It contains a unique Buffer RBZ that minimizes contamination from genomic DNA and protein. This kit efficiently isolates highly pure RNA from blood, cells, tissues, and plant samples in 1 h. The purified RNA is ready-to-use in downstream applications, such as RT-PCR and real-time RT-PCR, gene-chips assay, northern blot, poly A screening, in vitro transcript, and molecular cloning.

Important Note

The EasyPrep Cell & Bacteria RNAprep Purification Kit (Cat.no. DPT-BD30) is recommended for isolation of bacterial RNA.

Notes of preventing RNase contamination

1. Change gloves regularly. Bacteria on the skin could result in RNase contamination.
2. Use RNase-free plastic and tips to avoid cross contamination.
3. RNA must be stored or processed in RNase-free plastic or glassware. For wiping off the RNase, the glassware can be roasted at 150 °C for 4 h, whereas the plastic can be dipped in 0.5 M NaOH for 10 min, washed with RNase-free double-distilled water (ddH_2O) thoroughly, and sterilized.
4. Use RNase-free ddH_2O to confect solution (add DEPC into water in a 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).

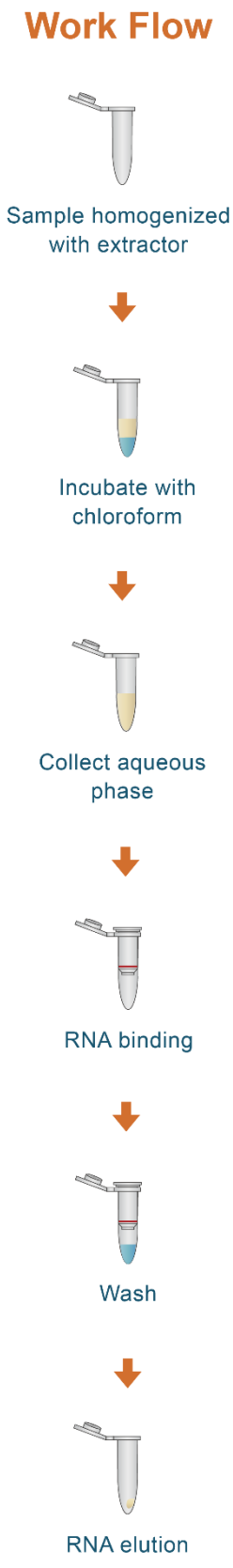
Kit Contents

Contents	DPT-BD19 (50 preps)
Buffer RBZ	60 ml
Buffer RBD	12 ml
Buffer RBW	12 ml
RNase-free ddH_2O	15 ml
RNase-free Spin Columns CR3 (with 2 ml tubes)	50
RNase-free Collection Tubes（1.5 ml）	50

Storage

Buffer RBZ should be stored at 2–8 °C; others should be stored at room temperature (15–25 °C).

Workflow


Work Flow
Sample homogenized with extractor
Incubate with chloroform
Collect aqueous phase
RNA binding
Wash
RNA elution

Protocol

Buffer RBD and Buffer RBW 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 RBZ after cutting leaves into pieces. This process is suggested to be finished within a minute. Use 1 mL Buffer RBZ per 100 mg leaves.
    b. Tissues (take rat liver as an example): Add 1 mL Buffer RBZ per 30–50 mg of liver sample. Homogenize the sample by using a power homogenizer. Typically, the volume of tissue sample should not exceed 10% of the volume of Buffer RBZ.
    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 before lysis (cells grown in a monolayer in cell culture flasks should always be trypsinized).
        i. **Method A**: To lyse cells directly. Add 1 mL Buffer RBZ 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 RBZ 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.

        ii. **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 × g for 5 min. Completely aspirate the supernatant.

        Note: Make sure 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 through centrifugation and remove the culture medium. Add 1 mL of Buffer RBZ per 5×10^6–10^7cells from animal, plant, or yeast, or 1×10^7 bacterial cells. Do not wash cells before adding Buffer RBZ to avoid increasing the chances of mRNA degradation. Samples from some yeast and bacteria might need to be homogenized by using a power homogenizer.
    e. Blood: Take fresh blood and add three volumes of Buffer RBZ. Mix thoroughly. (Recommended amount: 0.75 mL Buffer RBZ for 0.25 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), additional centrifugation might be required to remove insoluble material from the samples. Notably, the 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 homogenized part for the next step.

4. Add 200 µL of chloroform per 1 mL Buffer RBZ used for homogenization. Cap the tube securely and vortex for 15 s. Incubate for 3 min at room temperature.

Note: If vortex is not applicable, shake tube vigorously with 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 (precipitate 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 batches and centrifuge separately.

7. Add 500 µL Buffer RBD 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 500 µL Buffer RBW 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 RBW 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: Prolonged 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, and 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 might affect the recovery efficiency. Moreover, to obtain higher productivity, add the solution obtained from step 11 to the center of the membrane again, let the column stand for 1 min, and then centrifuge. Purified RNA should be

stored at −70 °C.