10. Add 350 μL Buffer RW1 to the Spin Columns RM, centrifuge at 12,000 rpm for 1 min, discard the waste liquid, and put the Spin Columns RM back into the collection tube.
11. Add 500 μL Buffer RW2 (check whether 100% ethanol has been added before use), to the Spin Columns RM , centrifuge at 12,000 rpm for 20 s. Discard the waste liquid, and put the Spin Columns RM back into the collection tube.
12. Repeat Step 11.
13. Centrifuge at 12,000 rpm for 2 min, discard the waste liquid. Leave the Spin Columns RM at room temperature for a few minutes and dry thoroughly.

    Note: The purpose of this step is to remove residual ethanol from the Spin Columns RM, which can interfere with subsequent enzymatic reactions (such as Enzyme digestion, PCR).
14. Place the Spin Columns RM in a new RNase-Free Centrifuge Tube, 30-50 μL of RNase-Free Water is added to the middle of the Spin Columns RM, leave it at room temperature for 1 min. Centrifuge at 12,000 rpm for 1 min, collect the RNA solution, and store the RNA at -70°C.

    Note: 1) The volume of RNase-Free Water should not be less than 30 μL, too small volume affects the recovery rate.

    2) If the RNA yield is to be increased, repeat step 14 with 30-50 μL of new RNase-Free Water.

    3) If the RNA concentration is to be increased, the obtained solution can be re-added into the Spin Columns RM and repeat step 14.

Version: 01/2024

Ultrapure RNA Kit (DNase I)

Cat. No. : CW0597S (50 preps)

Storage Condition: DNase I and 10× Reaction Buffer are stored at -20°C, TRIzon Reagent and TRIzon Pal™ are stored at 2-8°C away from light, and other components are stored at room temperature (15-30°C).

Components

Component	CW0597S (50 preps)
DNase I	1000 U
10×Reaction Buffer	1000 μL
TRIzon Reagent	60 mL
TRIzon Pal™	10 mL
Buffer RW1	40 mL
Buffer RW2（concentrate）	11 mL
RNase-Free Water	10 mL
Spin Columns RM with Collection Tubes	50
RNase-Free Centrifuge Tubes（1.5 mL）	50

Introduction

This kit is based on TRIzon improved column total RNA extraction kit. This product can extract total RNA from animal tissues, plant materials, various microorganisms and cultured cells. Firstly, the lysis solution fully lyses and homogenizes the sample, in the unique high salt state, RNA specifically binds to the silica matrix membrane, which reduces the protein contamination to a great extent and removes organic solvent contamination effectively. The RNA obtained is of better purity and quality. This product can quickly extract total RNA from various cells or tissues, each time can handle 30-50 mg of tissue or 5×10^6 cells, and can handle several different samples at the same time. If the RNA experiments that are very sensitive to trace DNA, the residual DNA can be removed by digestion on the column using RNase-Free DNase, and the extracted RNA can be directly applied to RT-PCR, Northern Blot, Dot Blot, in vitro translation and other experiments.

Precautions

1. To prevent RNase pollution, pay attention to the following aspects:

1.1 Use RNase-Free plastic products and tips to avoid contamination.

1.2 The solution should be prepared using RNase-Free water.

1.3 Operators should wear disposable masks and gloves, and change gloves routinely during the experiment.

2. The extracted samples should avoid repeated freeze-thawing, which will affect the yield and quality of RNA extraction.
3. If you find that the Trizon Reagent has a precipitation before use, you can place it in 56°C for a few minutes to solve the solubility.
4. Before using the first use, you should add 100% ethanol to the Buffer RW2 in accordance with the reagent bottle label.
5. All centrifugation steps are carried out at room temperature without special instructions, and all steps should be done quickly.

Protocol

1. Sample treatment

1.1 Tissue: 30-50 mg of tissue is fully ground in liquid nitrogen and then add to 1 mL of TRIzon Reagent, or 1 mL of TRIzon Reagent is added to the tissue sample and homogenize.

Note: The sample volume should not exceed 10% of the TRIzon Reagent volume.

1.2 Monolayer culture cells: Aspirate the culture solution and add the appropriate amount, 1 mL of TRIzon Reagent per 10 cm^2.

1.3 Cell suspension: Collect cells by centrifugation. Add 1 mL of TRIzon Reagent per 5×10^6 cells.

2. After adding TRIzon Reagent to the sample, blow repeatedly several times, make the sample fully cracked. Leave at room temperature for 5 min to completely separate the protein nucleic acid complex.
3. TRIzon PaI™ is added at a rate of 200 μL of TRIzon PaI™ per 1 mL of TRIzon Reagent, cover the tube, shake violently for 15 s and leave at room temperature for 2 min.
4. After centrifugation at 4°C at 12,000 rpm (~13,400×g) for 10 min, the samples will be divided into three layers: red organic phase, middle layer and upper colorless water phase. RNA is mainly in the upper water phase, and the upper water phase is moved to a new RNase-Free centrifuge tube (self-provided).
5. An equal volume of 70% ethanol (prepared with RNase-Free water) is added to the aqueous solution obtained, and the mixture is reversed.
6. Add all the solutions obtained in the previous step into Spin Columns RM with Collection Tubes. If the solution cannot be added at one time, it can be transferred several times. Centrifuge at 12,000 rpm for 20 s, discard the waste liquid, and put the adsorption column back into the collection tube.
7. Add 350 μL Buffer RW1 into Spin Columns RM, centrifuge at 12,000 rpm for 20 s, discard the waste liquid, and put the adsorption column back into the collection tube.
8. Preparation of DNase I mixture: Take 52 μL of RNase-Free Water, add 8 μL of 10×Reaction Buffer and 20 μL of DNase I (1 U/μL) to it, mix well, and make a final volume of 80 μL of reaction solution.
9. Add 80 μL DNase I mixture to the Spin Columns RM and incubate at 20-30°C for 15 min.