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. Add 200 μL TRIzon Pal ™ to each 1 mL of TRIzon Reagent, cover the tube, shake violently for 15 s, and leave at room temperature for 2 min.
4. Centrifuge at 12,000 rpm (~13,400 ×g) for 10 min at 4°C, the samples are 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. Add the same volume of 70% ethanol (prepared with RNase-Free water) to the obtained aqueous solution, mix it upside down.
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 700 μL Buffer RW1 into the adsorption column, centrifuge at 12,000 rpm for 20 s, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
8. Add 500 μL Buffer RW2 (check whether 100% ethanol has been added before use) to the adsorption column , 12,000 rpm centrifuge for 20 s, discard the waste liquid, and put the adsorption column back into the collection tube.
9. Repeat Step 8.
10. Centrifuge at 12,000 rpm for 2 min and discard the waste liquid. Place the adsorption column at room temperature for a few minutes and dry thoroughly.
    Note: The purpose of this step is to remove residual ethanol from the adsorption column, which will affect subsequent enzymatic reactions (enzyme digestion, PCR, etc.).
11. Put the adsorption column in a new RNase-Free centrifuge tube. 30-50 μL RNase-Free Water is added to the middle of the adsorption column. The adsorption column is placed at room temperature for 1 min and centrifuged at 12,000 rpm for 1 min. RNA is stored at -70°C to prevent degradation.
    Note: 1) The volume of RNase-Free Wate should not be less than 30 μL, too small volume will affect the recovery.
    2) To increase RNA yield, repeat step 11 with 30-50 μL of new RNase-Free Water.
    3) If the RNA concentration is to be increased, the solution obtained can be re-added to the adsorption column and repeat step 11.

Version: 02/2024

Ultrapure RNA Kit

Cat. No. : CW0581S (50 preps)
CW0581M (200 preps)

Storage Condition: TRIzon Pal™ and TRIzon Reagent should be stored away from light at 2-8°C, and other components are stored at room temperature (15-30°C).

Components

Component	CW0581S 50 preps	CW0581M 200 preps
TRIzon Reagent	60 mL	2×110 mL
TRIzon Pal™	10 mL	2×20 mL
Buffer RW1	40 mL	160 mL
Buffer RW2（concentrate）	11 mL	50 mL
RNase-Free Water	10 mL	50 mL
Spin Columns RM with Collection Tubes	50	200
RNase-Free Centrifuge Tubes（1.5 mL）	50	200

Introduction

This kit is based on TRIzon improved column total RNA extraction kit, the lysis solution fully lysed and homogenized samples, using a unique silicon matrix membrane adsorption technology, through the centrifugal adsorption column in the high salt state of high efficiency and specific binding of RNA in solution. At the same time, proteins, inorganic salt ions and organic impurities are removed effectively. Total RNA can be rapidly extracted from animal tissues, plant materials, various microorganisms and cultured cells, etc. It can process 30-50 mg of tissues or 5×10^6 cells each time, and it can process several different samples simultaneously. The RNA extracted by this kit can be directly used in RT-PCR, Northern Blot, Dot Blot, in vitro translation and other experiments.

Product features

1. High purity: Protein and other impurities are removed to the maximum extent, the extracted RNA can be directly used in various downstream experiments.
2. Large amount of extraction: The unique lysate formula can fully lysate cells or tissues, and the amount of RNA extraction up to 100 μg.
3. Fast: Few steps, simple operation, saving time.
4. Strong compatibility: Suitable for RNA extraction from a variety of animal and plant tissues and cells.

Reagents to be Supplied by user

70% ethanol (prepared with RNase-Free water), 100% ethanol.

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 Glassware should be sterilized at 180°C for 4 hours before use, plastic ware can be soaked in 0.5 M NaOH for 10 min, rinse thoroughly with water and then autoclaved.

1.3 The solution should be prepared using RNase-free water.

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

2. The extracted samples should avoid repeated freeze-thawing, which would 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.
6. If the downstream experiment is very sensitive to DNA, it is recommended that the RNA be treated with DNase I (Cat. No.: CW2090S) without RNase.

Protocol

1. Sample treatment

1.1 Plant tissue: Take fresh plant tissue in liquid nitrogen to fully grind or cut the plant tissue and directly in Trizon Reagent quickly grinds. Add 1 mL of TRIzon Reagent every 30-50 mg of tissue and mix well.

Note: Sample volume should generally not exceed 10% of the volume of TRIzon Reagent.

1.2 Take fresh or -70°C frozen animal tissues and cut them up as much as possible. 1 mL TRIzon Reagent is added every 30-50 mg of tissue, and homogenated by homogenizer. Or after grinding in liquid nitrogen add 1 mL TRIzon Reagent and mix well.

Note: Sample volume should generally not exceed 10% of the volume of TRIzon Reagent.

1.3 Monolayer cell culture: After the culture medium is sucked, appropriate amount of TRIzon Reagent can be added directly into the culture plate (1 mL of TRIzon Reagent for every 10 cm^2 area), and cells can be lysed by blowing repeatedly with sampler. Alternatively, after treatment with trypsin, cell solution is transferred to RNase-Free centrifuge tube, centrifuged at 300×g for 5 min, cell precipitation is collected, all supernatants are carefully absorbed, and 1 mL of TRIzon Reagent is added and mixed.

Note: 1) The number of cells collected should not exceed 1×10^7.

2) Dosage of TRIzon Reagent was determined by the area of culture plate, not by the number of cells. Insufficient dosage of TRIzon Reagent may result in DNA contamination in the extracted RNA.

3) Cell culture medium must be removed cleanly when collecting cells, otherwise it will lead to incomplete lysis and decrease of RNA production.

1.4 Cell suspension: Cell collection by centrifugation. Add 1 mL TRIzon Reagents for every 5×10^6-1×10^7 animal, plant, and yeast cells or for every 10^7 bacterial cells.

Note: 1) Do not wash cells before adding TRIzon Reagent to avoid RNA degradation.

2) Some yeast and bacterial cells may require homogenizer or liquid nitrogen grinding.

1.5 Blood treatment: Fresh blood is directly taken and added with 3 times the volume of TRIzon Reagent (0.25 mL whole blood is recommended and 0.75 mL TRIzon Reagent), fully shake and mix well.

1.6 Optional Steps: For samples with high content of protein, fat, polysaccharide or extracellular substances, such as muscle tissue, adipogenic tissue or plant tubers, centrifugation can be performed at 4°C and 12,000 rpm (~13,400×g) for 10 min after homogenization to remove insoluble substances. In this case, extracellular substances, polysaccharides and high molecular weight DNA are contained in the precipitation. And RNA is in the supernatant.