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4. The samples are centrifuged at 12,000 rpm at 4°C for 15 min. At this time, the samples are divided into three layers: the red organic phase, the intermediate layer and the upper colorless water phase. The RNA is mainly in the water phase, and the water phase (about 600 μL) is transferred to a new RNase-Free centrifuge tube (self-provided).
5. Add equal volume isopropanol to the aqueous solution, mix upside down and leave at room temperature for 10 min.
6. Centrifuge at 4°C at 12,000 rpm for 10 min and discard the supernatant.
7. The precipitation is washed with 75% ethanol (prepared with RNase-Free water). The precipitation is washed with 1 mL 75% ethanol for every 1 mL of TRIzon.
8. Centrifuge at 4°C at 12,000 rpm for 3 min. Carefully discard the supernatant and do not discard the RNA precipitate.

   Note: The remaining small amount of liquid can be centrifuged for a short time, and then sucked out with the pipette. Be careful not to discard the precipitation.
9. Leave at room temperature for 2-3 min to dry. The RNA is fully dissolved by adding 30-100 μL RNase-Free water, and the obtained RNA is stored at -70°C to prevent degradation.

   Note: The precipitation should not be too dry, so as not to dissolve.

TRIzon Reagent

Cat. No. : CW0580S (100 mL)
Storage Condition: Store this reagent at 2-8°C away from light.

Components

Component	CW0580S (100 mL)
TRIzon Reagent	100 mL

Introduction

TRIzon is a broad-spectrum reagent for extracting total RNA. The experimental operation is fast and convenient, the color is bright, and it is easy to layer. This reagent has a wide range of applications and can be used to extract total RNA from animal tissues, plant materials, various microorganisms and cultured cells. When the sample is fully cracked in TRIzon, the integrity of RNA can be ensured to the maximum extent. After centrifugation with trichloromethane, the solution is divided into three layers: the upper colorless aqueous phase, the middle layer and the lower red organic phase, and RNA is distributed in the upper layer. After collecting the supernatant, the total RNA can be recovered by isopropanol precipitation. The total RNA extracted has good integrity and no protein and DNA pollution, and can be used in a variety of routine molecular biology experiments, such as RT-PCR, Real-time RT-PCR, Northern Blot, Dot Blot, in vitro translation and so on.

Reagents to be Supplied by user

Trichloromethane, Isopropanol, 75% ethanol, RNase-Free water (newly opened or used for RNA extraction)

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 tools can be soaked in 0.5M NaOH for 10 minutes, washed thoroughly with water and sterilized under high pressure.

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 will affect the yield and quality of RNA extraction.
3. When using this product, you should wear protective articles, such as protective clothing, gloves, eye mask, face mask, etc. If you accidentally contact your eyes, flush them with plenty of water immediately and seek medical treatment. Note: Keep away from fire and heat sources when using.
4. After the sample is homogenized with TRIzon, if trichloromethane is not added immediately, it can be stored at -70°C for more than one month.
5. RNA precipitates stored in 75% ethanol can be preserved for one week at 2-8°C and for one year at -20°C. Since RNA has a short half-life and is easy to degrade, it is recommended to conduct follow-up experiments as soon as possible after extraction, such as reverse transcription into cDNA and Northern Blot.
6. If the downstream assay 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 tissues: Take fresh plant tissues and fully grind them in liquid nitrogen or cut up plant tissues and quickly grind them directly in TRIzon. Add 1 mL TRIzon to every 30-50 mg tissue and mix well.

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

1.2 Animal tissues: Fresh or frozen animal tissues stored at -70°C are cut up as far as possible, and 1 mL TRIzon is added into every 30-50 mg tissue, and homogenized by homogenizer. Or add 1 mL TRIzon after grinding in liquid nitrogen and mix well.

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

1.3 Monolayer cell culture: After removing the culture solution, an appropriate volume of TRIzon (1 mL TRIzon per 10 cm^2 area) can be added directly to the culture plate, and the cells can be lysed by repeated blowing with a sampler. After trypsin treatment, the cell solution is transferred to a RNase-Free centrifuge tube, centrifuged at 300 ×g for 5 min, cell precipitates are collected, all supernatants are discarded, and 1 mL TRIzon is added and mixed.

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

2) The volume of TRIzon is determined by the culture plate area, not by the number of cells. If the amount of TRIzon is insufficient, it may result in DNA contamination in the extracted RNA.

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

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

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

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

1.5 Blood treatment: Take fresh blood directly, add 3 times the volume of TRIzon (0.25 mL of whole blood is recommended to add 0.75 mL of TRIzon), fully shake and mix.

1.6 Optional steps: For samples with high content of proteins, fats, polysaccharides or extracellular substances, such as muscle tissue, adipocytes 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. At this time, extracellular substances, polysaccharides and high molecular weight DNA are contained in the precipitates. And RNA is in the supernatant.

2. After adding TRIzon into the sample, blow repeatedly several times to fully crack the sample. Leave at room temperature for 5 min to completely separate the protein nucleic acid complex.
3. Add trichloromethane to the above solution, add 0.2 mL trichloromethane to each use of 1 mL TRIzon, cover the tube, shake violently for 15 s, and leave at room temperature for 2-3 min.