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 pipette. Be careful not to discard the precipitation.
9. Leave at room temperature for 2-3 minutes to dry. The RNA is fully dissolved by adding 30-100 μL RNase-free water, and the resulting RNA is stored at -70°C to prevent degradation.
   Note: The precipitation should not be too dry, so as not to dissolve.

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

Version: 02/2024

TRIzon Pal™

Cat. No. : CW3166M (100 mL)

Storage conditions: 0-30°C store away from light

Components

Component	CW3166M
TRIzon Pal™	100 mL

Introduction

TRIzon Pal™ can be used with TRIzon Reagent (Cat. No.: CW0580S) to replace chloroform. It can repeatedly extract the pyrolysis system to remove proteins. Total RNA is extracted from animal tissues, plant materials, various microorganisms, and cultured cells. The sample is fully lysed in TRIzon to ensure the integrity of RNA to the maximum extent. After centrifugation with TRIzon Pal™, the solution is divided into three layers: the upper layer is colorless aqueous, mesosphere, and lower red organic phase, RNA distribution in the supernatant. After the supernatant layer is collected, total RNA can be recovered by isopropyl alcohol deposition. The extracted total RNA has good integrity, no protein or DNA contamination, and can be used in various routine experiments of molecular biology, such as RT-PCR, Real-time RT-PCR, Northern Blot, Dot Blot, in vitro translation, etc.

Reagents to be Supplied by user

TRIzon Reagent (Cat. No.: CW0580S), isopropanol, 75% ethanol, RNase-free water (newly opened or special for RNA extraction).

Precautions

1. Prevention of RNase pollution, should pay attention to the following aspects:
   1) Use RNase-free plastic products and tips to avoid cross contamination.
   2) Glassware should be dry baked at 180°C for 4 hours before use, plastic ware can be soaked in 0.5 M NaOH for 10 minutes, rinse thoroughly with water and autoclave.
   3) The solution should be prepared using RNase-free water.
   4) Operators should wear disposable masks and gloves, and change gloves frequently during the experiment.
2. The extracted samples should avoid repeated freeze-thawing, which would 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. 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.
5. If the downstream assay is very sensitive to DNA, it is recommended that the RNA be treated with DNase I (item number: CW2090S) without RNase.

Protocol

1. Handling of various materials

1a. 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.

1b. 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.

1c. Monolayer cell culture: After the culture medium is removed, appropriate amount of TRIzon can be directly added into the culture plate (1 mL TRIzon per 10 cm^2 area), and the cells can be repeatedly blown with the sampler to crack. 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 carefully absorbed, 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 TRNzol 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 medium must be removed cleanly when collecting cells, otherwise it will lead to incomplete lysis and decrease of RNA production.

1d. 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.

1e. 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.

1f. 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 minutes to completely separate the protein nucleic acid complex.
3. Add TRIzon Pal™ to the above solution, add 0.2 mL of TRIzon™ for every 1 mL of TRIzon used, cover the tube, shake vigorously for 15 s, and leave at room temperature for 2-3 min.
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 minutes.
6. Centrifuge at 4°C at 12,000 rpm for 10 min and discard the supernatant.