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TRIpure Total RNA Extraction Reagent

Introduction

TRIpure Total RNA Extraction Reagent is a reagent for total RNA extraction from various animal and plant, bacterial tissues/cells. It has strong cleavage ability to lyse cell and tissue samples in a short time and keep RNA intact in the sample. and effectively inhibit the degradation of RNA. The sample can be fully cleaved in the reagent. After centrifugation with chloroform, the solution forms a supernatant layer, an intermediate layer and an organic layer (lower red layer). The RNA is distributed in the upper aqueous phase, and the supernatant layer is collected. Total RNA can be recovered by precipitation of propanol. The extracted total RNA is high in purity and contains no protein or genomic DNA. It can be directly used for Northern, dot hybridization, mRNA purification, in vitro translation, RT-PCR, poly(A)+ selection, RNase protection analysis, and construction of cDNA libraries. A variety of molecular biology experiments.

In addition, after removal of the aqueous layer, the DNA and protein in the sample can also be successively reduced by precipitation. Ethanol precipitation can precipitate the DNA of the intermediate layer, and isopropanol can be added to the organic layer to precipitate the protein.

This product is simple and fast to operate, and all operations can be completed in one hour. And a small amount of tissue (50-100mg) and cells (5×10^6) and a large number of tissues (≥ 1g) and cells (> 10^7) have a good lysis effect.

Transportation and Storage

Ice bag transportation. The product is stored at 4℃ in the dark and is valid for one year.

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Attention

1) You need to self-supply chloroform, isopropyl alcohol (for new or imported RNA), 75% ethanol (DEPC water), DEPC and DEPC water.

2) Wear disposable clean gloves; operate in a separate clean area; avoid talking during operation to prevent contamination of the RNase in the sweat and saliva of the experimenter.

3) Please use RNase free experimental equipment, including the tip and centrifuge tube. The high temperature resistant material can be baked at 150 ° C for 4 hours to remove RNase, and other utensils to remove RNase can be considered to be soaked in 0.1% DEPC water overnight, then sterilized and dried. The solution needs to be prepared with DEPC water. Instruments and reagents for RNA experiments should be used exclusively and should not be used in other experiments. DEPC water is recommended to be stored after dispensing.

4) This product contains phenol, which is toxic and corrosive. If inhaled, in contact with skin, swallowed, etc., it can cause poisoning, burns and other bodily harm. Wear protective equipment such as protective clothing, gloves, eye masks, face masks, etc. when using this product. If you accidentally come into contact with your eyes, rinse immediately with plenty of water and go to hospital for treatment;

5) After the sample is homogenized with TRIpure, if it is not immediately added to chloroform for downstream experiments, it can be frozen at -70 ℃ for more than one month. The RNA precipitate stored in 75% ethanol was stored at 4 ° C for 1 week and stored at -20 ° C for 1 year.

6) RNA half-life is relatively short and easy to degrade. It is recommended to carry out subsequent experiments as soon as possible after extraction.

Dosage Reference

Table 1 The maximum sample size that can be fully lysed per 1 ml of TRIpure is as follows:

Adherent cell	10 cm^2Culture area
Suspended animal or plant cells or yeast cells	5 × 10^6-1×10^7

bacterial	10^7
Whole blood	50 μl
Animal tissue	30-100 mg
Plant tissue (low content of polysaccharides and polyphenols)	50-100 mg

*Excessive sample volume can result in insufficient lysis and a decrease in product purity.

Operation Steps

1. Sample processing

1.1 Sample homogenization

A. Adherent cells

Discard culture solution as much as possible, add 1 ml of TRIpure directly to a 3.5 cm-diameter plate and Pipette the lysate up and down several times.

【Note】:

1) Determine the amount of TRIpure required (1 ml per 10 cm^2) depending on the area of the plate rather than the number of cells.

2) When the amount of TRIpure added is insufficient, the extracted RNA may be contaminated with DNA.

3) Adherent cultured cells often cannot be completely detached from the culture flask (dish). This does not mean that the lysis is incomplete. At this time, the cell membrane has actually completely cleaved and RNA has been released, and subsequent experiments can be continued.

B. Suspended cells

Collect the cells by centrifugation, add 1 ml of TRIpure to every 5 x 10^6-1 x 10^7 cells, and pipetted repeatedly until no significant granules were present.

[Note]: Avoid washing cells before adding TRIpure, otherwise it will increase the possibility of mRNA degradation. Cracking certain yeasts and bacteria may require the use of a homogenizer.

C. Animal/plant organization

Fresh plant tissues or frozen tissue tissues at -70°C were thoroughly ground in liquid nitrogen, and the appropriate amount of TRIpure was added

according to Table 1. Or take fresh plant and animal tissue as much as possible, add the appropriate amount of TRIpure, homogenate for homogenization.

[Note]: The sample volume should generally not exceed 10% of the TRIpure volume.

1.2 Shake the homogenate sample igorously and allow to stand at room temperature for 5 minutes to completely dissociate the ribosome.

1.3 (Optional) Centrifuge at 12000 rpm for 10 min at 4℃ and remove the supernatant.

[Note]: If the sample contains more protein, fat, polysaccharide or muscle, the tuber nodules of the plant can be removed by centrifugation. The precipitate after centrifugation contains an extracellular membrane, a polysaccharide, and a high molecular weight DNA, and the supernatant contains RNA. When processing adipose tissue samples, the upper layer is a large amount of oil should be removed as much as possible, and a clear homogenate is taken for subsequent experiments.

2. Total RNA extraction

2.1 Add 1/5 volume of chloroform to the above lysate. Cap the tube cap tightly, shake vigorously for 15 seconds, and let stand for 2-3 minutes at room temperature.

2.2 Centrifuge at 12,000 rpm for 4-15 minutes at 10°C.

【Note】:

1) After centrifugation, the mixture can be divided into three layers: an upper layer of colorless water-like layer, an intermediate layer, and a lower layer of red organic phenol chloroform. RNA is present in the upper of colorless water-like layer.

2) The capacity of this part is about 50-60% of the total amount of added TRIpure. If extracted with 1 ml TRIpure, the upper layer is approximately 500-600 μl. It is recommended to draw 400-500 μl, do not suck too much, in order to prevent the absorption of the middle layer leading to genomic contamination.

3) red organic phenol chloroform and the intermediate layer are proteins and DNA. If necessary, please keep them and carry out relevant purification experiments.

2.3 Carefully pipette the upper layer into a new centrifuge tube and add an equal volume of isopropanol. Mix at room temperature for 10 min after inverting and mixing. (RNA precipitation is usually not visible before centrifugation. When the amount of extraction is large, a gelatinous precipitate forms on the tube side and the bottom of the tube after centrifugation.)

2.4 Centrifuge at 12,000 rpm for 10 minutes at room temperature or 4°C.

2.5 Carefully discard the supernatant and add 1 ml of 75% ethanol in DEPC water. Wash the tube cover and tube wall thoroughly, and flick the bottom of the tube to suspend the sediment. The pellet was washed with 1 ml of 75% ethanol per 1 ml of TRIpure.

2.6 12,000 rpm Centrifuge at room temperature or 4 ° C for 3 minutes, discard the supernatant, taking care not to lose RNA precipitation.

[Note]: The remaining small amount of liquid can be centrifuged briefly, then sucked out with a pipette tip, taking care not to abandon the sediment.

2.7 Leave at room temperature for 2-3 minutes and allow to dry. Add 30-100 ul of RNase free water (DEPC water). After complete dissolution, take a small amount of test and store at -70 °C.

3. Product testing

A. Integrity detection

1) Add 1 µl of RNA to the appropriate 10×DNA loading buffer and mix.

2) Perform 1% agarose gel electrophoresis. If you can see the clear three bands, it proves that the RNA integrity is good.

[Note]: If it is ordinary agarose gel electrophoresis, the position of 28S is about 2kb, and the position of 18S is about 1kb. The position of gel at different concentrations changes greatly.

B. Purity testing

The OD value at 260 nm and 280 nm was measured and OD_{260}/OD_{280} was calculated. The ratio of pure RNA should be between 1.9 and 2.2.