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Trizoe Reagent

Product Number: RNK0101

Shipping and Storage

Trizoe can be stably stored at room temperature for 12 months. Nevertheless, for optimal results, we recommend storing in an environment of 2-8°C.

Important Note

Toxic substances that come into contact with the skin or are accidentally swallowed can cause burns. Once in contact with the skin, wash immediately with a large amount of detergent and clear water. If you feel unwell, see a doctor and seek the correct treatment plan for phenol and other components.

Description

Trizoe reagent is a reagent that directly extracts total RNA from cells or tissues. It can maintain the integrity of RNA during cell fragmentation and lysis. After adding chloroform and centrifuging, the sample is divided into a water sample layer and an organic layer.RNA exists in the water sample layer. After collecting the water sample layer above, RNA can be reduced through isopropanol precipitation. After removing the water sample layer, the DNA and proteins in the sample can also be successively reduced by precipitation. Ethanol precipitation can precipitate DNA from the intermediate layer, while adding isopropanol to the organic layer can precipitate protein. Co purified DNA is very useful for standardizing RNA production between samples.

Whether it is human, animal, plant, or bacterial tissue, this method applies to small amounts of tissue (50-100mg) and cells (5×10^6) and a large number of tissues (≥ lg) and cells (>10^7) have good separation effects. The simplicity of Trizoe reagent operation allows for simultaneous processing of multiple samples. All operations can be completed within one hour. The total RNA extracted by Trizoe can avoid contamination of DNA and proteins. Therefore, it can perform RNA blotting analysis, dot hybridization, poly (A)+selection, in vitro translation, RNA enzyme protection analysis, and molecular cloning. If used for PCR, it is recommended to use amplification grade DNase I to process the extracted total RNA when two primers are located within a single exon.

Trizoe reagent can promote the precipitation of various RNAs of different genera and molecular weight sizes. For example, RNA agarose gel electrophoresis extracted from rat liver and stained with ethidium bromide showed many discontinuous high molecular weight bands (mRNA and hnRNA components) between 7 kb and 15 kb, and two dominant ribosomes~5 kb (28S) and~2 kb (18S) low molecular weight RNAs between 0.1 and 0.3 kb (tRNA, 5S). When the extracted RNA was diluted with TE, its A260/A280 ratio was>1.8.

Application

Suitable for rapid extraction of total RNA, DNA, and proteins from various animal and plant tissues/cells.

Note

1. When the dosage of Trizoe is less than 2ml, it is recommended to use clean disposable polypropylene material test tubes.
2. When the amount of Trizoe used is large, glass test tubes (Corex) or polypropylene test tubes can be used, and pre tested to ensure that the test tube can withstand the centrifugal force of 12000×g after adding Trizoe and chloroform. Do not use test tubes with cracks or damage.
3. Carefully balance the test tube before centrifugation.
4. Before centrifugation, the glass tube mouth must be covered with aluminum foil and sealed with paraffin film, while the polypropylene tube must be tightly covered.
5. Separate RNA samples from a small amount of tissue (1-10mg) or cells (10^2~10^4): Add 800μl of Trizoe to the tissue or cells. After the sample is cracked, chloroform is added and the extraction operation in step 2 is carried out. Before precipitating RNA with isopropanol, 5-10μg of RNA free glycogen was added as a carrier for the water sample layer. To reduce its viscosity, use a

26 syringe to aspirate twice before adding chloroform to cut off genomic DNA. Glycogen will remain in the water sample layer and co precipitate with RNA. It will not inhibit the synthesis of the first chain of reverse transcription reaction or PCR until it is concentrated to 4mg/ml.

6. After homogenization and before adding chloroform, the sample can be stored at -60~-70°C for at least one month. RNA precipitation (step 4, RNA rinsing) can be stored in 75% ethanol for at least one week at 2-8°C and for at least one year at -5-20°C.
7. The desktop centrifuge can achieve a maximum centrifugal force of 2.600×g. If the centrifugal time is extended to 30-60 minutes, the operations in steps 2 and 3 can be met.

Protocol

Note:When using Trizoe to extract RNA, gloves and eye protectors should be worn. Avoid contact with skin and clothing. Complete the operation in the chemical fume hood. Avoid respiratory inhalation. Unless otherwise specified, all operations should be carried out under conditions ranging from 15 to 30℃.

Items that were not provided with laboratory reagents:

1. Chloroform
2. Isopropanol
3. 75% ethanol (prepared with DEPC treated water)
4. RNA free water or RNA free water prepared with 0.5% SDS——Add water to a glass bottle without RNA enzyme and add DEPC to 0.1% (v/v). Leave overnight and autoclave. SDS solution must be prepared with DEPC treated and high-pressure sterilized water.

1. Homogenization

1.1. Tissue

Mix the tissue sample evenly with glass or a strong homogenizer, adding 1ml of Trizoe for every 50-100mg of tissue. The volume of the tissue sample during homogenization should not exceed 10% of the volume of Trizoe.

1.2. Monolayer grown cells

Add 1ml of Trizoe directly to a culture plate with a diameter of 3.5 cm to dissolve cells, and remove the cell lysate in batches through a pipette. Determine the required amount of Trizoe based on the area of the culture plate rather than the number of cells (add 1ml per $10cm^2$). When the amount of Trizoe is insufficient, it can lead to DNA contamination in the extracted RNA.

1.3. Suspended growing cells

Precipitate cells by centrifugation. Repeat blowing with a pipette in the Trizoe reagent to lyse cells. Add 1ml of Trizoe to every $5\sim10\times10^6$ animal cells, plant or yeast cells, or every 1×10^7 bacteria. Avoid washing cells before adding Trizoe,as it increases the likelihood of mRNA degradation. Rupture of certain yeast and bacteria may require the use of a homogenizer.

Option: When the sample is rich in protein, fat, polysaccharides, or extracellular substances such as muscle, adipose tissue, and plant tuber parts, an additional separation step may be required. After homogenization, centrifuge at 12.000×g for 10 minutes at 2-8°C to remove insoluble substances from the homogenate. The remaining precipitate contains extracellular membrane, polysaccharides, and high molecular weight DNA, while the upper layer of ultraplankton contains RNA In samples from adipose tissue, a large amount of fat floats on the top layer and should be removed. In each case, transfer the clear homogenate solution to a clean test tube, add chloroform, and continue with the following separation steps.

2. Separated Stage

Incubate the homogenate sample at 15-30°C for 5 minutes to completely decompose the ribosome. Add 0.2ml of chloroform to every 1ml of Trizoe. Cover the sample tube tightly, shake the test tube vigorously with your hand for 15 seconds, and incubate it at room temperature for 2-3 minutes. Freeze and centrifuge at a high speed of no more than 12000×g at 2-8°C for 15 minutes. After centrifugation, the mixture is divided into three layers: the lower phenol chloroform layer, the middle layer, and the upper colorless water sample layer. RNA exists without exception in the water sample layer. The capacity of the water sample layer is approximately 60% of the added Trizoe capacity.

3. RNA precipitation

Transfer the water sample layer to a clean test tube, and if you want to separate DNA and protein, the organic layer should also be retained. RNA was precipitated by mixing the water sample layer with isopropanol. At the initial homogenization, every 1ml of Trizoe corresponds to 0.5ml of isopropanol. Incubate the mixed sample at 15-30°C for 10 minutes and freeze centrifuge at a high speed of no more than 12.000×g at 2-8°C for 10 minutes. RNA precipitation is usually not visible before centrifugation, forming a colloidal sheet like precipitate that adheres to the wall and bottom of the test tube.

4. RNA rinsing

Remove the upper suspension. Wash RNA precipitation with 75% ethanol once, and add at least 1ml of 75% ethanol to every 1ml of Trizoe. Vortex oscillate the mixed sample and freeze it at a centrifugal force of no more than 7500×g at 2-8°C for 5 minutes.

5. Resolution of RNA

At the end of the operation, simply dry the RNA precipitate (air drying or vacuum drying for 5-10 minutes) and do not centrifuge dry the RNA in a vacuum tube. It is particularly important not to allow RNA precipitation to completely dry, which would greatly reduce its solubility. The A260/280 ratio of partially dissolved RNA samples is less than 1.6. Using the tip of a pipette, remove RNA free water or 0.5% SDS solution several times to dissolve RNA (avoid using SDS when RNA is to be used for enzyme cleavage reactions in the future). RNA can also be dissolved by 100% methicillin (ion removed) and stored at -70°C.

Problems and Solutions

Problems	Solutions
Expected RNA production per 1mg of tissue or 1×10^6cultured cells	1. Liver and spleen 6~10μg 2. Kidney 3~4μg 3. Skeletal muscles and brain tissue 1~1.5μg 4. Placenta 1~4μg 5. Epithelial cells(1×10^6 cultured cells) 8~15μg 6. Fibroblasts(1×10^6 cultured cells) 5~7μg
Low extraction yield	1. Sample homogenization or incomplete cracking. 2. Incomplete re dissolution of final RNA.
A260/A280 ratio<1.65	1. Dilute the RNA sample with water instead of TE buffer before measuring with a spectrophotometer. Low ion strength and low pH solutions will increase the light absorption value at 280nm. 2. The amount of Trizoe added during sample homogenization is too small. 3. After homogenization, the sample was not left at room temperature for 5 minutes. 4. The phenol layer is contaminated in the separated water sample layer. 5. Final RNA not completely dissolved.
RNA degradation	1. The tissue removed from the animal body was not immediately extracted or frozen. 2. The samples used for extraction, or RNA samples extracted, are stored at -5~-20°C instead of -60~-70°C. 3. Cells are dispersed through trypsin digestion. 4. Contamination of aqueous solution or test tube with RNA enzyme. 5. Formalin for agarose gel electrophoresis, pH lower than 3.5.
DNA contamination	1. The amount of Trizoe added during sample homogenization is too small. 2. The samples used for extraction include organic solvents (such as ethanol, DMSO), strong buffer solutions, or alkaline solutions.
Protein polysaccharides and polysaccharide contamination	The following improvements to the RNA precipitation method (step 3) can remove composite contamination from the extracted RNA. Taking every 1ml of TrizoeE during homogenization as an example, 0.25ml of isopropanol was added to the water sample layer, followed by 0.25ml of a high salt solution (0.8M sodium citrate and 1.2M NaCI). Mix the final solution well,

	centrifuge and continue with the aforementioned extraction operation. The improved precipitation method can effectively precipitate RNA while polysaccharides and proteoglycans remain in soluble form in the solution. For plants containing a large amount of polysaccharides, it is necessary to extract their RNA by combining the improved precipitation method with an additional centrifugation during initial homogenization (RNA extraction guidelines, optional solution).