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Trizoe LS Reagent

Product Number: RNK0201

Shipping and Storage

Trizoe LS Reagent can be stably stored at room temperature for 12 months. However, 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

The Trizoe LS Reagent reagent is a reagent that extracts total RNA directly from liquid samples derived from humans, animals, plants, yeast, bacteria, and viruses. 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.

This method has good separation effects on small amounts of tissue (50-100mg) and cells (5×10^6), as well as large amounts of tissue (≥lg) and cells ($>10^7$), whether from human, animal, plant, or bacterial tissues. The simplicity of Trizoe LS Reagent reagent operation allows for simultaneous processing of multiple samples. All operations can be completed within one hour. The total RNA extracted by Trizoe LS Reagent 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.

The Trizoe LS Reagent reagent can promote the precipitation of various RNAs of different species 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 between 7kb and 15kb, two dominant ribosomes~5kb (28S) and~2kb (18S), and low molecular weight RNA between 0.1 and 0.3kb (tRNA, 5S). When the extracted RNA is diluted with TE, its A260/A280 ratio is ≥ 1.8.

Application

Suitable for rapid extraction of total RNA, DNA, and proteins from liquid or solid samples from various sources

Note

1. When the dosage of Trizoe LS Reagent is less than 2ml, it is recommended to use a clean disposable polypropylene material test tube.
2. When the dosage of Trizoe LS Reagent is high, 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 LS Reagent 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 (102-104): Adjust the sample volume to 0.25ml and

add 0.75ml of Trizoe LS Reagent to the tissue or cells. After the sample is cracked, add chloroform and perform the extraction operation in step 2. 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, aspirate twice with a No.26 syringe 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 at 2-8°C for at least one week, and at -5-20°C for at least one year.
7. A desktop centrifuge can achieve a maximum centrifugal force of 2600×g. If the centrifugal time is extended to 30-60 minutes, the operations in steps 2 and 3 can be met.

RNA extraction steps

Note: When using Trizoe LS Reagent to extract RNA, gloves and eye protection 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 at 15-30°C

Items required for the experiment but not provided:

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. Biological liquid

Add 0.75ml of Trizoe LS Reagent to every 0.25ml of liquid samples (serum, plasma, cerebrospinal fluid, etc.), and blow the liquid sample several times with a sampling gun to help lyse the cells in the sample. Add at least 0.75ml of Trizoe LS Reagent to every 5~10×10^6 cells. The final volume ratio between Trizoe LS Reagent and liquid samples is always 3:1.

1.2. Organization

Mix the tissue sample well with glass or a strong homogenizer, and add 0.75ml of Trizoe LS Reagent to every 50-100mg of tissue or 0.25ml of tissue suspension. Generally, the volume of 50-100mg tissue should be less than 0.25ml. If the volume of the tissue sample is less than 0.25ml, add sterilized water to adjust the volume of the tissue sample to 0.25ml to ensure a volume ratio of 3:1.

1.3. Single layer growing cells

Add 0.3ml-0.4ml of Trizoe LS Reagent directly to a culture plate with a diameter of 3.5 cm to dissolve cells, and blow with a sampling gun to help fully lyse cells. The required amount of Trizoe LS Regant is determined based on the area of the culture plate rather than the number of cells (add 0.3-0.4ml per 10cm^2). There is no need to add water to the lysate, as the residual culture medium attached to the culture plate has been fully diluted with Trizoe LS Reagent.

1.4. Suspended growing cells

Settle cells by centrifugation. Repeatedly blow and beat with a pipette in the Trizoe LS Reagent reagent to lyse cells. Add 0.75ml of Trizoe LS Reagent to every 106 animal cells, plant or yeast cells, or every5~10×10^6 bacteria. Adjust the sample volume to 0.25ml using sterilized water as in step 1.2. Avoid washing cells before adding Trizoe LS Regant, as it increases the likelihood of mRNA degradation. Rupture of certain yeast and bacteria may require the use of a homogenizer.

Optional solution: 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 12000×g for 10 minutes under conditions of 2-8°C to remove insoluble substances from the homogenization. The remaining precipitate contains extracellular membrane, polysaccharides, and high molecular weight DNA, while the upper layer of superplankton

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 ribosomes. Add 0.2ml of chloroform to every 0.75ml of Trizoe LS Reagent. Cover the sample tube tightly, shake the test tube vigorously by hand for 15 seconds, and incubate it at 30°C for 2-15 minutes. Freeze and centrifuge at high speed with a centrifugal force not exceeding 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 70% of the added Trizoe LS Reagent 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 0.75ml of Trizoe LS Regant corresponds to 0.5ml of isopropanol. Incubate the mixed samples at 15-30°C for 10 minutes and freeze centrifuge at a maximum centrifugal force of 12.000×g for 10 minutes at 2-8°C. 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. **Rinsing of RNA**

Remove the upper suspension. Wash RNA precipitation with 75% ethanol once, and add at least 1ml of 75% ethanol to every 0.75ml of Trizoe LS Reagent. 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. **Re dissolution 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. Use the tip of a pipette to remove RNA free water or 0.5% SDS solution several times to dissolve RNA, and incubate at 55-60°C for 10 minutes (avoid using SDS when RNA is to be used for enzyme cleavage reactions in the future). RNA can also be dissolved again by 100% methicillin (excluding ions) and stored at -70°C.

Problems and Solutions

Problems	Solutions
Expected RNA production per 1ml liquid sample or 1mg tissue or 1×10^6 cultured cells	1. Whole blood of humans and animals 15-20μg 2. Human lymphocytes (approximately 7×10^7 white blood cells)60~70μg 3. Liver and spleen 6~10μg 4. Kidney 3~4μg 5. Skeletal muscle and brain tissue 1~1.5μg 6. Placenta 1~4μg 7. Epithelial cells(1×10^6 cultured cells) 8~15μg 8. 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 LS Reagent added during sample homogenization is too small. 3. The sample was not left at room temperature for 5 minutes after sluicing. 4. The phenol layer is contaminated in the separated water sample layer. 5. The final RNA did not completely dissolve.

RNA degradation	1. The tissue taken 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. Water solution or test tube contamination with RNA enzymes. 5. The pH of formalin used for agarose gel electrophoresis is lower than 3.5.
DNA contamination	1. The amount of Trizoe LS Reagent 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 Trizoe as an example during homogenization, 0.25ml of isopropanol was added to the water sample layer, followed by 0.25ml of 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 multiple centrifugations during initial homogenization (RNA extraction guidelines, optional approach).