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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

This product contains phenol, which is toxic and corrosive. If inhaled, in contact with the skin, swallowed, etc., it can cause poisoning, burns, and other bodily injuries. When using this product, protective equipment such as protective clothing, gloves, eye masks, face shields, etc. should be worn. If accidentally touched, immediately rinse with plenty of water and seek medical treatment.

Description

Trizoe Reagent is a broad-spectrum total RNA extraction reagent. The experimental operation is fast and convenient, with bright colors and easy layering. This reagent has a wide range of applications and can extract total RNA from animal tissues, plant materials, various microorganisms, and cultured cells. This method has good separation effects on small amounts of tissues (50-100mg) and cells (5×10^6), as well as large amounts of tissues (≥ 1g) and cells (>107). The sample can be fully lysed in TRIpure while maximizing the integrity of RNA. After centrifugation with chloroform, the solution will be divided into three layers: the upper colorless aqueous phase, the middle layer, and the lower red organic phase, with RNA distributed in the supernatant layer. After collecting the supernatant, total RNA can be recovered by precipitation with isopropanol. The extracted total RNA has good integrity, no protein or DNA contamination, and can be used for various routine molecular biology experiments, such as RT-PCR Real-time RT-PCR、Northern blot、Dot Blot、 External translation, etc.

Trizoe reagent can promote the precipitation of various RNAs of different species and molecular weights. 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, two dominant ribosomes~5 kb (28S) and~2 kb (18S), and low molecular weight RNA between 0.1 and 0.3 kb (tRNA, 5S). When the extracted RNA is diluted with TE, its A260/A280 ratio is ≥ 1.8. Note that for ordinary agarose gel electrophoresis, the position of 28S is about 2kb, and 18S is about 1kb. The position of gel with different concentrations varies greatly.

Note

1. After homogenizing the sample with Trizoe Reagent, if chloroform is not added immediately, it can be placed at -70°C for more than a month. RNA precipitation stored in 75% ethanol can be preserved for one week at 2-8°C- It can be stored for 1 year at 20°C. RNA has a relatively short half-life and is easily degraded. It is recommended to conduct subsequent experiments as soon as possible after extraction, such as reverse transcription into cDNA, Northern Blot, etc.
2. If downstream experiments are highly sensitive to DNA, it is recommended to use DNase I and RNase free kit (RNK4501) to treat RNA.
3. Self prepared reagents: chloroform, isopropanol (newly opened or specifically for RNA extraction), 75% ethanol (prepared with DEPC treated water) RNase free water or DEPC treated water.

Protocol

Note:Wear gloves and eye protection when extracting RNA with Trizoe. Avoid contact with skin and clothing. Complete the operation in the chemical fume hood. Avoid inhalation through the respiratory tract. Unless otherwise specified, all operations should be carried out under conditions of 15-30 ℃.

1. Homogenate

1.1. **Plant tissue:** Take fresh plant tissue and grind it thoroughly in liquid nitrogen or cut it into pieces and quickly grind it in

Trizoe Reagent. Add 1ml Trizoe Reagent to every 50-100mg of tissue and mix well.

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

1.2. **Animal tissue:** Fresh or frozen animal tissue at -70 ℃ should be cut into small pieces as much as possible, and 1ml of Trizoe Reagent should be added every 30-100mg of tissue for homogenization treatment using a homogenizer. Or grind in liquid nitrogen and add 1ml of Trizoe Reagent to mix well.

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

1.3. **Single layer culture of cells:** After removing as much residual culture medium as possible, add 1ml of Trizoe Reagent directly to a 3.5-cm diameter culture plate to cover and repeatedly blow and lyse the cells. Determine the required amount of Trizoe Reagent based on the area of the culture plate rather than the number of cells (1ml per $10cm^2$). When the amount of Trizoe reagent is insufficient, it can lead to DNA contamination in the extracted RNA.

Note: Cells cultured on the wall often cannot completely detach from the culture bottle (dish), which does not mean incomplete lysis. At this point, the cell membrane has actually completely ruptured and released all RNA. Continue with the procedure.

1.4. **Cell suspension:** Collect cells by centrifugation. Use a pipette to repeatedly blow and lyse cells in the Trizoe Reagent reagent. Add 1ml of Trizoe Reagent every 5-10×10^6 animal cells, plant or yeast cells, or every 1 × 107 bacteria. Wash cells should be avoided before adding Trizoe Reagent, as it increases the likelihood of mRNA degradation. Cracking certain yeast and bacteria may require the use of a homogenizer.

1.5. **Blood:** We recommend using our company's Trizoe LS Reagent (RNK0201), which is a Trizoe Reagent specifically designed for whole blood or liquid samples. LS stands for Liquid Sample. Equivalent to Invitrogen's TRIzol LS.

2. Shake the homogenized sample vigorously and leave it at room temperature for 5 minutes to completely dissociate the ribosomes.

3. **Optional steps:** Centrifuge at 12000 rpm for 10 minutes at 4°C and collect the supernatant.

If the sample contains a high amount of protein, fat, polysaccharides or muscle, plant tubers and nodules can be removed by centrifugation. The centrifuged precipitate contains cell outer membrane, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA. When processing samples of adipose tissue, a large amount of oil should be removed from the upper layer. Take the clarified homogenate for the next step.

4. Add 0.2ml chloroform to every 1ml of Trizoe Reagent. Cover the tube tightly, shake vigorously for 15 seconds, and let it sit at room temperature for 2-3 minutes.

5. Freeze centrifuge at high speed at 4°C and 12000 rpm for 10-15 minutes. After centrifugation, the mixture is divided into three layers: the lower layer is a red organic phenol chloroform layer, the middle layer, and the upper layer is a colorless water sample layer. RNA is present in the aqueous layer without exception. The capacity of the water sample layer is approximately 50-60% of the capacity of the added Trizoe Reagent. The organic layer and intermediate layer consist of protein and DNA. If extraction is required, please contact us for the extraction method.

6. Transfer the water sample layer to a clean centrifuge tube and add an equal volume of isopropanol. After reversing and mixing, let it stand at room temperature for 10 minutes. RNA precipitation is usually not visible before centrifugation, but forms a gel like precipitate on the tube side and bottom after centrifugation.

7. Centrifuge at room temperature or 4°C at 12000 rpm for 10 minutes and discard the supernatant.

8. Add 75% ethanol to wash the precipitate. Wash the precipitate with 1ml of 75% ethanol for every 1ml of Trizoe reagent used.

9. Centrifuge at room temperature or 4°C at 12000 rpm for 3 minutes, discard the supernatant, and be careful not to lose the RNA precipitate.

Note: The remaining small amount of liquid can be briefly centrifuged and then sucked out with a gun tip, being careful not to discard the sediment.

10. Leave at room temperature for 2-3 minutes and air dry. Add 30-100μl RNase free water, dissolve RNA thoroughly, and store the obtained RNA at -70°C to prevent degradation.

Note: Do not dry the sediment excessively to avoid difficulty in dissolution.