Trizol

T751379

Storage: Store at Room temperature

Introduction:

Trizol is a ready-to-use reagent for the isolation of high-quality total RNA from cell and tissue samples of animal, plant or bacteria origin. With this product, RNA up to 15kb can be isolated. small RNAs such as microRNAs can also be isolated, but should be precipitated overnight at -70℃ after adding isopropanol.

The RNA isolated with Trizol is free of DNA and protein contamination. In general, the A260/280 value of the isolated RNA in DEPC water is 1.8-2.0. This product maintains the integrity of the RNA due to highly effective inhibition of RNase activity during sample homogenization and cell lysis. With this product, 5-15μg of RNA can be obtained per million cells, 1-10μg of RNA can be obtained per milligram of tissue. The yield varies depending on cells and tissues. With this product, RNA isolation from two samples can be completed in approximately one hour. Isolated RNA can be used in Northern Blot analysis, Dot Blot hybridization, mRNA purification, in vitro translation, RNase protection assay, molecular cloning, RT-PCR, microarray analysis, RNA-seq, and other applications that require high-quality RNA.

Usage method:

1. Cell lysis or tissue homogenization.

a. For adherent cells

Aspirate the culture medium and add 1ml of Trizol per 10cm^2 culture area. Typically, 1ml of Trizol per well in six-well plates and 0.5ml of Trizol per well in 12-well plates. Shake 3-5 times and pipette 2-3 times to ensure complete lysis, then transfer to a new centrifuge tube.

b. For suspension cells

Collect the cells by centrifugation and discard the supernatant. Add 1ml of Trizol for every 5-10 $\times 10^6$ cells from plant or yeast origin, or 1×10^7 bacterial cells, and pipette or vortex to ensure complete lysis. For some yeast and bacteria that are not lysed completely, use a homogenizer to ensure complete lysis.

c. For tissues

Cut the tissue into small pieces, add 1ml of Trizol per 50mg-80mg of tissue, and homogenize using a homogenizer. To ensure the integrity of RNA, it is recommended to freeze the tissue with liquid nitrogen and homogenize well at low temperature before adding Trizol.

Note: For cells or tissues with high protein, polysaccharide, or lipid content, lysis with Trizol may result in insoluble or greasy floating matter. In this case, centrifuge at 12,000×g at 4℃ for 10 minutes and then transfer the supernatant into a new centrifuge tube.

2. Incubate at room temperature for 5 minutes to allow complete lysis of the sample.

3. Add 0.2ml of chloroform per 1ml of Trizol, securely cap the tube, then vortex or shake vigorously for 15 seconds. Incubate at room temperature for 2-3 min.
4. Centrifuge at 12,000×g at 4℃ for 15 minutes. Transfer the colorless upper aqueous phase containing total RNA into a new tube, approximately 0.5-0.55 ml per ml of Trizol.
5. Add 0.5 ml of isopropanol to the aqueous phase, per ml of Trizol used for lysis. Mix by inverting the tube several times, and incubate at room temperature for 10 minutes. To extract small RNA such as microRNA, we recommend incubating at -70℃ overnight.
6. Centrifuge at 12,000×g at 4℃ for 10 minutes and discard the supernatant.
7. Add 1 ml of 75% ethanol (prepared with DEPC-treated water) per ml of Trizol used for lysis, vortex or invert the tube to mix well.
8. Centrifuge at 7,500×g at 4℃ for 5 min and discard the supernatant. Centrifuge briefly again (>5,000 rpm, 1 sec) and carefully remove the supernatant with a micropipette.
9. Vacuum or air dry the RNA pellet slightly, then dissolve the RNA in 20μl of DEPC-treated water. Proceed to downstream applications or store the RNA at -70℃. Do not over dry the RNA pellet. Otherwise, it will be extremely difficult to dissolve and the A260/280 value of the RNA will be less than 1.6.

Precautions:

1. Chloroform, isopropanol, DEPC, 75% ethanol (prepared with DEPC-treated water), and DEPC-treated water are required, but not provided in this product.
2. All centrifuge tubes, tips and solutions to be used must be free of RNAase. For consumables, heat at 150℃ for 4 hours or soak in 0.01% DEPC overnight followed by sterilization and drying. The solution should be prepared with DEPC-treated water. Add 0.01% (v/v) diethylpyrocarbonate (DEPC) to ddH_2O or Milli-Q water overnight, and sterilize to make DEPC-treated water.
3. Total RNA extraction from frozen cells or tissues is generally less effective than extraction from fresh cells or tissues.
4. Wear disposable gloves and a disposable mask during the whole process of RNA isolation to prevent RNase contamination.
5. Trizol contains the toxic phenol. Take cautions to avoid contact with skin or inhalation. To prevent splashing into eyes, wear goggles during the operation. In case of direct contact with Trizol, flush immediately with plenty of detergent and water and seek medical help if necessary.
6. This product is for R&D only. Not for drug, household, or other uses.
7. For your safety and health, please wear a lab coat and disposable gloves during the operation.