TIANGEN®

TRNzol Universal Reagent

For isolation of high quality total RNA from cell and tissue samples

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TRNzol Universal Reagent

Cat. no. 4992730

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Introduction

TRNzol Universal Reagent is developed based on regular TRNzol Reagent with the addition of indicator. It has better lysis ability and higher sensitivity. It could be used to isolate total RNA from samples like virus, bacteria, fungus, animal, plant tissue and body fluid. It maintains the integrity of RNA while disrupting cells and dissolving cell components during sample homogenization.

TRNzol Universal Reagent can be applied to isolate RNA from both small amount of samples (50-100 mg tissue or 5×10^6 cells) and large amount of samples (≥1 g tissue or $\geq 10^7$ cells) of human, animal, plant or bacteria, within one hour. DNA and protein contamination is eliminated thoroughly from RNA products. Isolated RNA can be used in Northern Blot, Dot Blot, poly(A) selection, *in vitro* translation, RNase protection assay and molecular cloning.

Storage

TRNzol Universal Reagent should be stored protected from light at 2-8°C for up to 12 months.

Important Notes

1. Before the addition of chloroform, homogenized samples in TRNzol Universal Reagent can be stored at –70°C for at least one month.
2. The RNA can be stored in 75% ethanol for at least 1 year at -20°C, or at least 1 week at 2-8°C.

Notes of preventing RNase contamination

1. Change gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-Free plastic and tips to avoid cross contamination.
3. RNA can be protected in TRNzol Universal Reagent. But RNA must be stored or processed in RNase-Free plastic or glassware. To wipe off RNase, the glassware can be heated at 150°C for 4 hours, while plastic can be dipped in 0.5 M NaOH for 10 min, washed by RNase-Free ddH_2O thoroughly, and sterilized.
4. Use RNase-Free ddH_2O to confect solution. (Add DEPC into water in clean glass container to a final concentration of 0.1% (v/v). Incubate overnight and autoclave for 15 min to remove any trace of DEPC.)

Protocol

Reagents to be supplied by user: chloroform, isopropanol, RNass-Free ddH_2O, 75% ethanol (in RNase-Free ddH_2O)

1. Homogenizing samples.
   a. Plant tissues (take leaves as an example): Place fresh leaves in liquid nitrogen and grind thoroughly with a mortar and pestle, or grind in TRNzol Universal Reagent after cutting leaves into pieces. This process is suggested to be finished within one minute. Use 1 ml TRNzol Universal Reagent per 100 mg leaves.
   b. Animal tissues (take rat liver as an example): Add 1 ml TRNzol Universal Reagent per 30-50 mg of liver sample. Homogenize sample using a power homogenizer. Usually, the volume of tissue sample should not exceed 10% of the volume of TRNzol Universal Reagent.
   c. Adherent Cells (do not use more than 1×10^7 cells): Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. (Cells grown in a monolayer in cell-culture flasks should always be trypsinized.)

1) Method A: To lyse cells directly. Add 1 ml TRNzol Universal Reagent directly to the cells in the culture dish per 10 cm^2 of culture dish surface area. Pipette the lysate up and down several times.

Note: the volume of TRNzol Universal Reagent should be determined according to the surface area instead of the number of cells. An insufficient volume can result in DNA contamination of isolated RNA.

2) Method B: To trypsinize and collect cells. Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.10–0.25% trypsin in PBS. After the cells detaching from the dish or flask, add medium containing serum to inactivate the trypsin, transfer the cells to an RNase-Free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 × g for 5 min. Completely aspirate the supernatant.

Note: Make sure that the supernatant has been completely removed. Residual medium could lead to incomplete lysis of cells and reduce yield of RNA.

d. Suspension Cells: Harvest cells by centrifugation and remove culture medium. Add 1 ml of TRNzol Universal Reagent per 5 × 10^6- 10^7 cells from animal or plant. Do not wash cells before addition of TRNzol Universal Reagent to avoid increased chance of mRNA degradation.

e. Blood and supernatant of virus culture medium: Take fresh blood and supernatant of virus culture medium, and add three volumes of TRNzol Universal Reagent. Mix thoroughly. (Recommended amount: 0.6 ml TRNzol Universal Reagent for 0.2 ml whole blood)

2. Incubate homogenized samples at 15-30°C for 5 min, to permit complete dissociation of the nucleoprotein complex.

3. *Optional step: Centrifuge the sample at 12,000 rpm (~13,400 × g) for 10 min at 4°C. Transfer the supernatant to a fresh micro-centrifuge tube.*

Note: When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional centrifugation may be required to remove insoluble material from the samples. RNA remains in the upper aqueous phase after centrifugation. However, when dealing with fat tissue, the upper phase is a lipid layer that should be discarded. Retain the clean homogenizing part for next step.

4. Add 0.2 ml chloroform per 1 ml TRNzol Universal Reagent used for homogenization. Cap the tube securely and vortex for 15 sec. Incubate for 3 min at room temperature.

   Note: If vortex is not applicable, shake tube vigorously by hand for 2 min.

5. Centrifuge the sample at 12,000 rpm (~13,400 × g) for 15 min at 4°C. The mixture separates into a lower pink phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Pipette the aqueous phase out into a new tube. (If isolation of DNA and protein is desired, ask TIANGEN for protocols.)
6. Add equal volume of 100% isopropanol to the aqueous phase. Mix thoroughly and incubate at room temperature for 10 min.
7. Centrifuge at 12,000 rpm (~13,400 × g) for 10 min at 4°C. Remove the supernatant from the tube, leaving only the RNA pellet. The RNA is often invisible prior to centrifugation, and after centrifugation it will appear as a gel-like pellet on the side and bottom of the tube.
8. Wash the pellet, with at least 1 ml of 75% ethanol (in RNase-Free ddH_2O) per 1 ml of TRNzol Universal Reagent used in the initial homogenization.
9. Centrifuge the tube at 10,000 rpm (~9,391 × g) for 5 min at 4°C. Discard the wash. Centrifuge briefly to collect residual liquid and pipette them out without drawing the pellet.
10. Air dry the RNA pellet for 2-3 min. (Do not allow the RNA to dry completely, because the pellet can lose solubility.) According to the requirement, add 30-100 µl RNase-Free ddH_2O, resuspend the RNA pellet completely by passing the solution up and down repeated through a pipette tip.

Expected yields

The table below presents typical yields of RNA from various starting materials.

Starting Material	RNA Yield
Plant leaf	100-500 µg / g leaves
Animal tissue	6-10 µg / mg liver tissues
Cultured cell	5-10 µg / 10^6 cells
Blood	3-5 µg / ml human whole blood

Troubleshooting

Comments	Suggestions
Low yield of RNA	A. Incomplete homogenization or lysis of samples. B. Final RNA pellet was incompletely redissolved.
Low $A_{260/280}$ (< 1.65)	A. RNA is dissolved in ddH_2O instead of TE. The A_{280} value increases in low ion concentration and low pH value. B. Sample was homogenized in an insufficient volume of TRNzol Universal Reagent. C. Homogenized samples had not been incubated at RT for 5 min. D. Interphase/organic phase pipetted up with aqueous phase. E. Final RNA pellet was incompletely redissolved.
RNA is degraded	A. Samples were not immediately processed or frozen after collection. B. Isolated RNA preparations were stored at the -5– -20°C, instead of in -60 – -70°C. C. Cells were injured during trypsinization. D. RNase hasn't been eliminated from reagents or plastic ware. E. The PH value of formamide used in electrophoretic is lower than 3.5.
DNA contamination	A. Sample was homogenized in an insufficient volume of TRNzol Universal Reagent. B. Residual organic reagents (like ethanol, DMSO, etc), buffer components or strong alkaline components in sample.
Protein contamination or polysaccharide contamination	A. High concentration of protein or polysaccharide in sample. B. The amount of starting sample is too large. C. Interphase/organic phase pipetted up with aqueous phase.