

Trizol (Total RNA Extractor Reagent)

T669996

Storage 2-8°C. Store in the dark.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

Trizol (Total RNA Extractor Reagent) is a reagent designed for the direct extraction of total RNA from cells or tissues. It maintains the integrity of RNA during the disruption and lysis of cells. Upon the addition of a Chloroform substitute/chloroform and subsequent centrifugation, the sample separates into an aqueous phase, an interphase, and an organic phase. RNA is present in the aqueous phase. After collecting the upper aqueous phase, RNA can be recovered by isopropanol precipitation. This method is effective for both small and large amounts of tissues and cells, regardless of whether they are from humans, animals, plants, or bacteria. The simplicity of Trizol Reagent allows for the simultaneous processing of multiple samples. All operations can be completed within one hour. Total RNA extracted with Trizol is free from DNA and protein contamination and can be used for RNA blotting analysis, dot hybridization, poly(A)+ selection, in vitro translation, RNase protection analysis, and molecular cloning. When used for PCR, it is recommended to treat the extracted total RNA with amplification-grade DNase I if both primers are located within a single exon. Trizol Reagent facilitates the precipitation of various types of RNA with different molecular weights across species. For example, RNA extracted from rat liver, when subjected to agarose gel electrophoresis and stained with ethidium bromide, shows numerous discontinuous high molecular weight bands between 7kb and 15kb (mRNA and hnRNA components), two major ribosomal bands with molecular weights of approximately 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 A_{260}/A_{280} ratio is ≥ 1.8 .

Product component

T669996	Component	100 ml	Storage
T669996A	TRzol	100 ml	2-8°C.
T669996B	Chloroform substitute	10 ml	2-8°C. Store in the dark.

Notes: Toxic substances may cause burns if they come into contact with skin or are accidentally ingested. In case of skin contact, immediately wash the affected area with plenty of detergent and water. If discomfort occurs, seek medical attention and obtain proper treatment for phenol and other components.

Reagents to Prepare Separately

Isopropanol, 75% ethanol (prepared with RNase-Free water), RNase-Free water (add DEPC to 0.1% (v/v) in RNase-free glass bottles, let stand overnight, and autoclave).

Procedure

1. Sample Pretreatment

- (1) Plant tissue (e.g., leaf RNA extraction): Grind fresh leaves thoroughly in liquid nitrogen or directly in TRzol reagent after cutting into small pieces, with grinding completed within 1 minute. Use 1ml TRzol per ~100mg leaf tissue.
- (2) Animal tissue (e.g., rat liver RNA extraction): For fresh or -70°C frozen tissue, add 1ml TRzol per 30-50mg tissue and homogenize. The sample volume should generally not exceed 10% of the TRzol volume.
- (3) Adherent cells: Add 1ml TRzol directly to a 3.5cm culture dish to lyse cells, and transfer the cell lysate with a pipette. The amount of TRzol is determined by the culture dish area (1ml per 10cm²) rather than cell count. Insufficient TRzol may lead to DNA contamination in the extracted RNA.
- (4) Suspension cells: Pellet cells by centrifugation and lyse them by pipetting up and down in TRzol. Add 1ml TRzol per 5-10×10⁶ animal, plant, or yeast cells, or per 1×10⁷ bacteria. Avoid washing cells before adding TRzol, as this may increase the likelihood of mRNA degradation. Homogenizers may be required to disrupt certain yeast and bacterial cells.

2. Separation Phase

Incubate the homogenized sample at 15-30°C for 5 minutes to completely dissociate nucleoprotein complexes. (Optional step: Centrifuge at 4°C, 12,000rpm (~13,400×g) for 10 minutes and collect the supernatant. Note: If the sample contains high levels of protein, fat, polysaccharides, or tissues like muscle or plant nodules, centrifuge to remove the precipitate. The precipitate contains cell membranes, polysaccharides, and high-molecular-weight DNA, while the supernatant contains RNA. For adipose tissue samples, remove the upper lipid layer and use the clear homogenate for the next step.)

Add 0.1ml Chloroform substitute per 1ml TRzol. Tighten the tube cap, shake vigorously by hand for 15 seconds, and incubate at room temperature for 2-3 minutes. Centrifuge at 4°C, 12,000rpm (~13,400×g) for 15 minutes. The mixture will separate into three layers: a lower organic layer, an intermediate layer, and an upper colorless aqueous layer. RNA is present in the upper aqueous layer, which typically constitutes ~60% of the initial TRzol volume.

3. RNA Precipitation

Transfer the aqueous layer to a new centrifuge tube (retain the organic and intermediate layers if DNA and protein separation is desired). Precipitate RNA by mixing the aqueous layer with isopropanol.

Add 0.5ml isopropanol per 1ml TRzol and mix thoroughly. Incubate the mixture at 15-30°C for 10 minutes, then centrifuge at 4°C, 12,000rpm (~13,400×g) for 15 minutes. The RNA precipitate is usually invisible before centrifugation but forms a gel-like pellet attached to

the tube wall and bottom after centrifugation.

4. RNA Washing

Remove the supernatant and wash the RNA precipitate 1-2 times with 75% ethanol (prepared with RNase-Free water), using at least 1ml of 75% ethanol per 1ml of TRzol. Vortex to mix and centrifuge at $\leq 7,500\times g$ for 5 minutes at 2–8°C.

5. RNA Redissolution

Air-dry the RNA precipitate at room temperature; do not dry it in a vacuum centrifuge. Importantly, avoid complete drying of the RNA precipitate, as this will significantly reduce its solubility. Partially dissolved RNA samples may have an OD_{260}/OD_{280} ratio <1.6 . Dissolve the RNA by pipetting RNase-Free water multiple times and store at -70°C.

Precautions:

1. Isolation of RNA samples from small amounts of tissue (1-10mg) or cells (10^2 - 10^4 cells): Add 800 μ l of TRzol to the tissue or cells. After the sample is lysed, add the Chloroform substitute and proceed with the extraction steps. Before precipitating RNA with isopropanol, add 5-10 μ g of RNase-free glycogen as a carrier for the aqueous phase. To reduce viscosity, pass the sample through a 26-gauge needle twice before adding chloroform to shear genomic DNA. Glycogen will remain in the aqueous phase and co-precipitate with RNA. It will not inhibit the synthesis of the first strand in reverse transcription reactions or PCR until it is concentrated to 4mg/ml.
2. Sample storage: The sample can be stored at -60°C or -70°C for at least one month before homogenization and addition of the Chloroform substitute (R749970). RNA precipitate dissolved in 75% ethanol can be stored at 2-8°C for at least one week and at -5 to -20°C for at least one year.
3. Safety: When extracting RNA with TRzol, wear gloves and eye protection. Avoid contact with skin and clothing. Perform the operation in a chemical fume hood. Avoid inhalation through the respiratory tract. Unless otherwise specified, maintain the room temperature between 15-30°C.