Total RNA Extraction Reagent (TRIzol®)

Catalog: RK30129

Size： 50 mL/ 100 mL

Components：

Components	Catalog	Size1	Size2
Total RNA Extraction Reagent (TRIzol®)	RM30129	50 mL	100 mL

Product Description

Total RNA Extraction Reagent (TRIzol®) rapidly extracts total RNA from a variety of biological samples, including (but not limited to) animal cells, tissue, plant materials, various microorganisms, and cell culture. This method is effective for small sample sizes for tissues (50-100 mg) and cells (5×10^6) as well as a large tissue (≥ 1 g) and cell samples (>10^7). Samples are adequately lysed in Total RNA Extraction Reagent while maximizing RNA integrity. Centrifugation with chloroform separates the solution into 3 layers: the aqueous phase (colorless, containing RNA), the interphase (containing DNA), and the lower organic phase (red, containing proteins). Total RNA can be recovered from the aqueous phase via precipitation with isopropanol. The total RNA extracted is intact and free from protein and DNA contamination.

Total RNA Extraction Reagent (Trizol®) facilitates the precipitation of various RNAs of various molecular weights, dependent upon source sample. For example, RNA extracted from rat liver displays discrete high molecular weight bands (mRNA and hnRNA components) between 7 and 15 kb, dominant ribosomal DNA bands between ~5 kb and ~2 kb (28S and 18S), and low molecular weight RNAs between 0.1 and 0.3 kb (tRNA, 5S) through gel electrophoresis. When extracted RNA is diluted with TE, A260/A280 ratio is ≥ 1.8.

Storage Conditions

The reagent is stable for 12 months at room temperature. For best results, it is recommended to store at 2-8°C. Occasional particles in the reagent may appear and will not affect reagent efficiency.

Precautions

This product contains phenol, which is toxic and corrosive. Poisoning, burns, or other bodily injuries can result if inhaled, swallowed, allowed to contact skin, etc. Please wear protective devices, such as protective clothing, gloves, goggles, face masks, etc., when using this product. In case of accidental contact, rinse immediately with plenty of water and go to hospital for treatment.

After homogenization with Total RNA Extraction Reagent, if chloroform is not added immediately, the

sample may be stored at -70°C for more than 1 month. RNA pellets immersed in 75% ethanol can be stored for 1 week at 2-8°C and 1 year at -20°C. RNA has a relatively short half-life and is easy to degrade; when possible, keep samples on ice throughout preparation. Perform subsequent experiments as soon as possible after extraction to avoid the effects of degradation.

Scope of Application

Total RNA Extraction Reagent can be used to generate RNA for use in various conventional molecular biology protocols, such as RT-PCR, Northern blot, Dot Blot, *in vitro* translation, etc.

Operational Instructions

Note: Gloves and goggles should be worn at all times. Perform operation in chemical fume hood. Avoid contact of reagents with skin and clothing. Avoid respiratory inhalation. Avoid sample contamination with RNase from hands and other sources. All operations should be performed at room temperature (15-30°C) unless otherwise specified.

Experimental Method

Users must prepare their own chloroform, isopropanol (freshly opened or dedicated for RNA extraction), 75% ethanol (formulated in DEPC-treated water), RNase free water or DEPC-treated water, and RNase-free sample tubes.

1. Homogenization

A. **Plant tissues**: Cryogrind fresh plant tissues thoroughly by snap-freezing in liquid nitrogen. Cut large pieces of tissue if needed, avoiding thawing of sample during preparation, or mechanically disrupt fresh or difficult-to-lyse samples. Add 1 mL of Total RNA Extraction Reagent per 50-100 mg of tissue and mix well. Note: The sample volume should generally not exceed 10% of the volume of Total RNA Extraction Reagent.

B. **Animal tissues**: Using cryopreserved (at -70°C) or freshly prepared animal tissues, add 1 mL of Total RNA Extraction Reagent per 30-100 mg tissue, and homogenize with a homogenizer. Alternatively, for highest RNA integrity, after cryogrinding samples in liquid nitrogen, 1 mL of Total RNA Extraction Reagent may be added and mixed well. Note: The sample volume should generally not exceed 10% of the volume of Total RNA Extraction Reagent.

C. **Monolayer culture cells**: After removing the residual culture medium as much as possible, add 1 mL of Total RNA Extraction Reagent directly to a 3.5 cm culture plate, and repeatedly pipette up and down to lyse the cells. The amount of Total RNA Extraction Reagent required (1 mL per 10 cm^2) is determined based on the area of the culture plate rather than the number of cells. Insufficient amount of Total RNA Extraction Reagent can lead to DNA contamination in the extracted RNA.

   Note: Adherent culture cells often cannot be completely detached from the culture flask (culture dish). With immersion in Total RNA Extraction Reagent, the cell membrane will completely rupture and release all RNA, regardless. After all the culture medium is aspirated, add 1 mL of Trizol® per 10 square centimeters of cells; add 1 mL of Trizol® to each well of a 6-well plate, or add 0.5 mL of Trizol ® to each well of a 12-well plate. Shake 3-5 times, pipette the cells up and down 2-3 times to ensure complete lysis, and then transfer into a centrifuge tube.

D. **Cell suspension:** Collect cells by centrifugation. Repeatedly pipette up and down in Total RNA Extraction Reagent to lyse the cells. Add 1 mL of Total RNA Extraction Reagent per 5-10 × 10^6 animal,

plant or yeast cells, or per 1 × 10^7 bacteria. Washing of the cells should be avoided before adding Total RNA Extraction Reagent, as this increases the possibility of mRNA degradation. Homogenizers are recommended for certain yeasts and bacteria that are difficult to rupture.

2. The homogenized samples should be vigorously shaken and left at room temperature for 5 minutes to allow complete dissociation of nucleosomes.
3. Optional step: Centrifuge at 12,000 rpm (~13,400 x g) for 10 minutes at 4°C and collect the supernatant.

   Note: If the sample contains a lot of protein, fat, polysaccharide or muscle, plant tuberous nodules can be removed by centrifugation. The centrifuged pellet contains extracellular membrane, polysaccharides, and high molecular weight DNA, and the supernatant contains RNA. When handling samples of adipose tissue, the upper layer is a large amount of oils and fats that should be removed. The clear homogenate should be taken for next step.
4. Add 0.2 mL of chloroform per 1 mL of Total RNA Extraction Reagent. Cap the tube tightly, shake vigorously for 15 seconds, and allow it to stand at room temperature for 2-3 minutes.
5. Centrifuge at 12,000 rpm (~13,400 x g) for 10-15 minutes at 4°C. After centrifugation, the mixture separates into 3 layers: a lower red organic phenol chloroform layer, a middle layer, and an upper colorless aqueous layer. RNA is present in the aqueous layer. The volume of the aqueous layer is approximately 50-60% of the volume of the Total RNA Extraction Reagent added. (The organic and middle layers are proteins and DNA).
6. Transfer the aqueous layer into a clean centrifuge tube and add an equal volume of isopropanol. Mix well by inversion and allow to stand at room temperature for 10 minutes.

   Note: RNA pellets are usually not visible prior to centrifugation, and gelatinous precipitates are formed on the side and bottom of the tube after centrifugation.
7. Centrifuge at 12,000 rpm (~13,400 x g) at room temperature or 4°C for 10 minutes and discard the supernatant.
8. Wash the pellets using 1 mL of 75% ethanol per 1 mL Total RNA Extraction Reagent.

   Note: Pipette up and down until the RNA pellets are gently suspended, but do not disperse.
9. Centrifuge at 12,000 rpm (~13,400 x g) at room temperature or 4°C for 3 minutes and discard the supernatant. Take care not to discard the RNA pellets.

   Note: The remaining small amount of liquid may be briefly centrifuged and then aspirated with a tip, taking care not to disturb the RNA pellets.
10. Allow to air dry at room temperature for 2-3 minutes. Add 30-100 µL of RNase free water to dissolve the RNA thoroughly. The resulting RNA should be stored at -70°C to prevent degradation.

    Note: Do not excessively dry the pellets to avoid being difficult to dissolve.

Determination of RNA concentration and purity

1. RNA concentration is determined using OD values and calculated from OD260/OD280. The quality of RNA can be preliminarily judged based on the ratio of OD260/OD280, which typically falls between 1.6 and 2.0. It is additionally recommended to examine RNA integrity by conventional RNA agarose electrophoresis.
2. To confirm RNA integrity, run 1 mg RNA from the sample to perform 1% agarose gel electrophoresis. Stain with ethidium bromide and visualize under ultraviolet light. 28S RNA (commonly at ~5 kb position) should be approximately twice as bright as 18S RNA (commonly at ~2 kb position), and sometimes 5S RNA with 0.1-0.3 kb is visible.