TRAzol Reagent

Catalog No.: R1021 (20 ml) , R1022 (100 ml)

Kit Contents

Component	R1021	R1022
TRAzol Reagent	20 ml	100 ml

Materials supplied by the users:

- Chloroform
- Isopropyl alcohol
- 75% ethanol (in RNase-free water)
- RNase-free water or 0.5% SDS solution [To prepare RNase-free water, add diethylpyrocarbonate (DEPC) to deionized water, and the final concentration is 0.01% (v/v). Treating overnight and then be autoclaved. The SDS solution must be prepared using DEPC-treated, autoclaved water.]

WARNING: TRAzol Reagent is corrosive, do not contact with skin directly or swallowing. If contact with skin, wash immediately with plenty of detergent and water. If you feel unwell, please seek for medical advice (show label when possible).

Description

TRAzol Reagent is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method. During sample homogenization or lysis, TRAzol Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation. Precipitation with ethanol yields DNA from the interphase, and an additional precipitation with isopropyl alcohol yields proteins from the organic phase.

Copurification of the DNA may be useful for normalizing RNA yields from sample to sample.This technique performs well with small quantities of tissue (50-100 mg) and cells (5×10^6), and large quantities of tissue (≥1 g) and cells (>10^7) of human, animal, plant, or bacterial origin.The simplicity of the TRAzol Reagent method allows simultaneous processing of a large number of samples. The entire procedure can be completed in one hour. Total RNA isolated by TRAzol Reagent is free of protein and DNA contamination.

Downstream Applications

Purified RNA is free of impurities and enzyme inhibitors, and have an $A_{260/280}$=1.8-2.0, is suitable for applications such as:

- Reverse transcription PCR (RT-PCR)
- Real-time quantitative PCR (qPCR)
- Northern blotting
- Nuclease protection assays
- RNA amplification for microarray analysis
- cDNA library preparation after poly(A)+ selection

Features

- Stable yield
- Reliable performance of high-quality purified total RNA in downstream applications

Storage

Store at 2-8˚C, protect from light, and is stable for up to 12 months when stored properly.

Important Notes

Precautions for Preventing RNase Contamination

RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA.

- Ensure that no RNases are introduced into the sterile solutions of the kit.
- Only use sterile, disposable RNase-free pipet tips and microcentrifuge tubes.
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin. Change gloves frequently, particularly as the protocol progresses from crude extracts to more purified material.
- In the presence of TRAzol Reagent, RNA is protected from RNase contamination. Downstream sample handling requires that nondisposable glassware or plasticware be RNase-free. Glass items can be baked at 150˚C for 4 hours, and plastic items can be soaked for 10 minutes in 0.5M NaOH, rinsed thoroughly with water, and be autoclaved.
- Always use proper microbiological aseptic techniques when working with RNA.

Recommended volume of TRAzol Reagent

10 cm^2 adherent cells	1 ml
10^7 suspension cells	1-2 ml
100 ul white cells	2 ml
50-100 mg ordinary tissue	1 ml
50-100 mg special tissue(liver, spleen, bone or cartilage)	2 ml
15-100 mg plant tissue	1 ml

Protocol

1. Sample processing
   - **Tissues**

   Tissue from animal or plant (either fresh or frozen at -70 ˚C until use) can be processed by freezing with liquid nitrogen and grinding into powder using mortar and pestle. Homogenize tissue samples in 1 ml **TRAzol Reagent** per 50-100 mg tissue. Tissue homogenizer or rotor-stator mixer also can be used.
   - **Adherent Cells**

   Lyse cells directly in a culture dish by adding 1 ml of **TRAzol Reagent** to the dish and passing the cell lysate several times through a pipet tip. The amount of TRAzol Reagent required is based on the culture dish area (1 ml per 10 cm^2) but not on the number of cells present.
   - **Suspension Cells**

   Harvest cells and pellet cells by centrifugation. Use 1 ml of **TRAzol Reagent** per 5–10×10^6 animal, plant, or yeast cells, or per 1×10^7 bacterial cells. Lyse cells by repetitive pipetting up and down. Do not wash cells before addition of TRAzol Reagent to avoid any mRNA degradation. Disruption of some yeast and bacterial cells may require a homogenizer.

2. Incubate at room temperature (15-30˚C) for 5 min.

3. **Optional** • Centrifuge at 12,000 rpm for 5 min at 4˚C, transfer the supernatant to a new RNase-free microcentrifuge tube. This step can eliminate protein, lipid, polysaccharide, musle or plant fibre.

4. Add 200 µl (1/5 volume of TRAzol Reagent) **chloroform**, mix by vortexing for 15 sec, incubate at room temperature for 3 min.

5. Centrifuge the sample at 12,000 rpm for 10 min at 4 ˚C. Transfer the colorless upper phase to a new RNase–free tube.
   - After centrifugation, the mixture separates into 3 layers: yellow organic phase, an interphase, and a colorless upper aqueous phase which contains the RNA.

6. Add the same volume of **isopropyl alcohol**, mix well. Incubate at room temperature for 10 min.

7. Centrifuge at 12,000 rpm for 10 min at 4˚C, discard the supernatant.

8. Add 1 ml **75% ethanol** (in RNase-free water), do not stir the precipitate, gently inverting the tube several times to wash the tube. Centrifuge at 12,000 rpm for 2 min at 4˚C, discard the supernatant. **Repeat this step**.

9. Pipet and eliminate residual ethanol, then open the tube to air dry for 5-10 min. Add 30-100 µl **RNase-free Water** (DEPC-treated water or 0.5% SDS solution) and mix thoroughly. The tube contains the purified RNA. **Store RNA at -70˚C.**
   - Avoid SDS when RNA will be used in subsequent enzymatic reactions.
   - Incubate at 50˚C for 10-15 min will accelerate dissolution.

Product Use Limitations

This product is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.