DONGSHENG BIOTECH

TRaZOL, reactivo para aislar RNA 100ml

Description

The TRAzol RNA Purification Kit provides a simple, reliable, and rapid method for isolating high–quality total RNA from a wide variety of samples, including animal and plant cells and tissue, bacteria, and yeast. The kit utilizes the strong lysis capability of TRAzol Reagent. 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. Total RNA isolated by TRAzol Reagent is free of protein and DNA contamination. It can be used for Northern blot analysis, dot blot hybridization, poly (A)+ selection, in vitro translation, RNase protection assay, and molecular cloning.

Features
The most classic formula
The most widely used
The most stable yield

Storage: Store at 2-8°C, protect from light for up to 12 months.

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.
Always wear disposable gloves. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases. Practice good microbiological technique to prevent microbial contamination.
Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any nondisposable items (such as automatic pipettes) can be rich sources of RNases.

Recommended volume of TRAzol on different starting materials
10 cm2 adherent cells
1 ml
107 suspension cells
1-2 ml
100 µl white cells
2 ml
50-100 mg ordinary tissue
1 ml
50-100 mg special tissue(live, spleen, bone or cartilage)
2 ml
15-100 mg plant tissue
1 ml

Protocol
Lysate Preparation with TRAzol Reagent
Use TRAzol Reagent to prepare lysates from various sample types as described below.

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 a fine powder using a mortar and pestle. Homogenize tissue samples in 1 ml TRAzol Reagent per 50–100 mg tissue using a tissue homogenizer or rotor-stator.

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 cm2) and not on the number of cells present.

Suspension Cells

Harvest cells and pellet cells by centrifugation. Use 1 ml of the TRAzol Reagent per 5–10 × 106 animal, plant, or yeast cells, or per 1 × 107 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.

Phase Separation

Following cell or tissue lysis (previous protocol), perform the following steps to isolate the RNA.

1. Incubate the lysate with TRAzol Reagent (previous protocol) at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.
2. Add 0.2 ml chloroform per 1 ml TRAzol Reagent used. Shake the tube vigorously by hand for 15 seconds.

Note: Vortexing may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to the presence of DNA.

3. Incubate at room temperature for 2–3 minutes.
4. Centrifuge the sample at 12,000× g for 5-10 min at 4°C.

Note: After centrifugation, the mixture separates into a lower, red phenol–chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA.

5. Transfer of the colorless, upper phase containing the RNA to a fresh RNase–free tube.
6. Add an equal volume of Isopropyl alcohol. Invert the tube to disperse any visible precipitate that may form after adding ethanol.
7. Centrifuge at 12,000× g for 15 seconds at room temperature.

RNA Wash

Discard the supernatant, add l ml 75% Ethanol (in DEPC-treated water), do not stir the precipitate, gently invert the tube several times to wash the tube, centrifuge at 12,000 x g for 2 min at 4 °C, discard the ethanol, repeat the step again.

Redissolving the RNA

Dry the tube containing the RNA precipitate 2–5 minutes, do not centrifuge or heat to dry, or the RNA will be difficult to dissolve. Add appropriate volume of Rnase-free water to dissolve the precipitate, pipetting if it is necessary to completely dissolve the RNA. Store at -80℃.

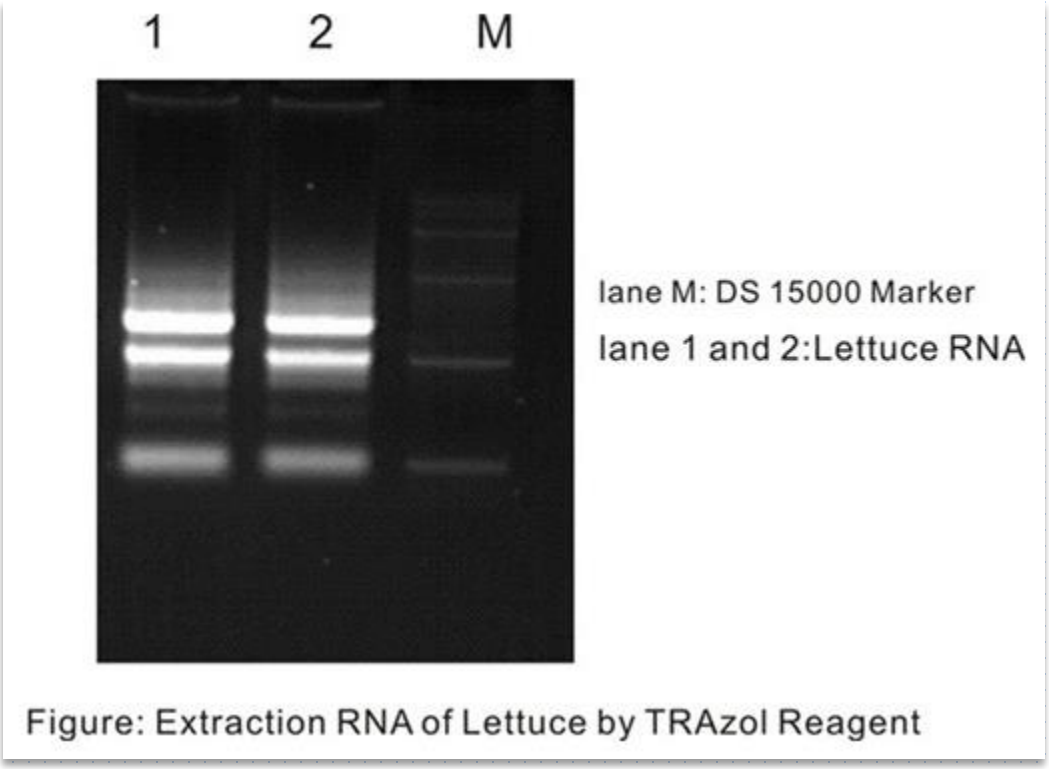

Figure: Extraction RNA of Lettuce by TRAzol Reagent