TriEx Reagent

Cat No. TRI-M-100
Size: 100 ml
Store at 4°C

MOLEQULE-ON®

Description

MOLEQULE-ON TRiEx 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, TRI-EX 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. Co-purification 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 TRI-EX Reagent method allows simultaneous processing of a large number of samples. The entire procedure can be completed in one hour. Total RNA isolated by TRI-EX 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. For use in the polymerase chain reaction (PCR), treatment of the isolated RNA with amplification grade DNase I is recommended when the two primers lie within a single exon.

Reagents and Equipment Required but Not Provided:

Chloroform, Isopropyl Alcohol, 75% Ethanol (in RNA-free water), RNase-free water

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.

1. 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.
2. 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 non-disposable items (such as automatic pipettes) can be rich sources of RNases.
3. In the presence of TRI-EX Reagent, RNA is protected from RNase contamination. Downstream sample handling requires that non-disposable 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 0.5 M NaOH, rinsed thoroughly with water, and autoclaved.

Recommended Volume of TRI-EX on Different Starting Materials

10 cm^2 adherent cells	1 ml
10^7 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

1. Homogenization

Use TRI-EX 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 TRI-EX 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 TRI-EX Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette. The amount of TRI-EX Reagent added is based on the area of the culture dish (1 ml per 10 cm^2) and not on the number of cells present. An insufficient amount of TRI-EX Reagent may result in contamination of the isolated RNA with DNA.

Suspension Cells

Pellet cells by centrifugation. Lyse cells in TRI-EX Reagent by repetitive pipetting. Use 1 ml of the reagent per 5-10 × 10^6 of animal, plant or yeast cells, or per 1 × 10^7 bacterial cells. Washing cells before addition of TRI-EX Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

OPTIONAL: An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and tuberous parts of plants. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 × g for 10 minutes at 2 to 8°C. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

2. Phase Separation

Incubate the homogenized samples for 5 minutes at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 1 ml of TRI-EX Reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at room temperature for 2 to 3 minutes. Centrifuge the samples at 12,000 × g for 5-10 minutes at 4°C. Following centrifugation, the mixture separates into a lower yellow, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRI-EX Reagent used for homogenization.

3. RNA Precipitation

Transfer the aqueous phase to a fresh tube, and save the organic phase if isolation of DNA or protein is desired. Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRI-EX Reagent used for the initial homogenization. Mix well by inverting the tube several times. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at 12,000 × g for 5-10 minutes at 4°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

4. RNA Wash

Discard the supernatant, add l ml 75% Ethanol (in RNA-free water), do not stir the precipitate, gently inverting 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.

5. Dissolving the RNA

At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 minutes). Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an A260/280 ratio < 1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60°C. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be re-dissolved in 100% formamide (deionized) and stored at -70°C.

WARNING: Toxic in contact with skin and if swallowed. Causes burns. After contact with skin, wash immediately with plenty of detergent and water. If you feel unwell, seek medical advice.