Ver. 24031

ORN01

TRI Reagent

For the extraction of total RNA from cells, fungus, tissue and plant samples.

Origin

ISO 13485:2016 ISO 9001:2015

TRI Reagent

(ORN01)

Introduction

TRI Reagent is a quick and convenient reagent for use in the simultaneous isolation of RNA, DNA, and protein. Successful isolations from human, animal, plant, yeast, bacterial, and viral samples can be obtained. A convenient single-step liquid phase separation results in the simultaneous isolation of RNA, DNA, and protein. This procedure is an improvement of the single-step method reported by Chomczynski and Sacchi for total RNA isolation. TRI Reagent performs well with large or small amounts of tissue or cells and many samples can be simultaneously extracted.

This product is a mixture of guanidine thiocyanate and phenol in a monophase solution which effectively dissolves DNA, RNA, and protein on homogenization or lysis of tissue sample. After adding chloroform and centrifuging, the mixture separates into 3 phases: an aqueous phase containing the RNA, the interphase containing DNA, and an organic phase containing proteins. Each component can then be isolated after separating the phases. 1mL of TRI Reagent is sufficient to isolate RNA, DNA, and protein from 50–100mg of tissue, 5–10x10^6 cells, or 10cm^2 of culture dish surface for cells grown in monolayer.

This is one of the most effective methods for isolating total RNA and can be completed in only 1 hour starting with fresh tissue or cells. The procedure is very effective for isolating RNA molecules of all types from 0.1–15kb in length. The resulting RNA is intact with little or no contaminating DNA and protein. Reagent maintains the integrity of the RNA due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization. This RNA can be used for Northern blots, mRNA isolation, *in vitro* translation, RNase protection assay, cloning and polymerase chain reaction (PCR).

The DNA is in the interphase and phenol phase, which forms after the addition of chloroform or 1-bromo-3-chloropropane to the TRI Reagent (Sample Preparation, step 2). After precipitation and multiple washes, the DNA is dissolved in 8mM NaOH. The solution is neutralized and the DNA is ready for analysis. The resulting DNA is suitable for PCR, restriction enzyme digestion, and Southern blotting. After precipitating the DNA with ethanol (DNA Isolation, step 1), the proteins can be removed from the phenol/ethanol supernatant. The isolated material can be probed for specific proteins by Western blotting.

Reagents Required but Not Provided

RNA Isolation

- Chloroform
- 100% Isopropanol
- 75% Ethanol
- RNase-free water of 0.5% SDS

DNA Isolation

- Chloroform
- 100% Ethanol
- 75% Ethanol
- 0.1M sodium citrate in 10% ethanol
- 8mM NaOH
- HEPES

Protein Isolation

- Chloroform
- Isopropanol
- 100% Ethanol
- 0.3M Guanidine hydrochloride in 95% ethanol
- 1% SDS

Procedures

1. Sample Preparation

Sample type	Starting material per 1mL of TRI Reagent
Tissues	20–50mg of tissue • Add 2mL TRI Reagent to tissue in which RNA can be difficult to extract (liver, spleen, bone, and cartilage)
Cells grown in monolayer	1×10^5–1×10^7 cells grown in monolayer in a 3.5cm culture dish ($10cm^2$)
Cells grown in suspension	5–10×10^6 cells from animal, plant, or yeast origin or 1×10^7 cells of bacterial origin
Peripheral Blood Mononuclear Cells (PBMC)	Isolated from 5mL whole blood by density gradient centrifugation

Notes:

a) **For RNA extraction from plant samples, it is recommended to use 100-150mg per 1mL of TRI Reagent.**

b) **Fungus:**

- Harvest 1-5×10^9 cells by centrifugation and remove culture medium. Add 600µL Buffer LY (not supplied), 100µL β-Mercaptoethanol and 10µL Lyticase 10U/µL (Cat. No.: ORT410, not supplied) to the pellet.

Note: The volume of Buffer LY, Lyticase and β-Mercaptoethanol will vary with cell number and type of cells.

- Incubate the sample at room temperature for 30 minutes. Occasionally mix the content by inverting the tube. Centrifuge the sample at 4,000 rpm for 5 minutes and remove the supernatant. Add 1mL of Buffer RZ to the pellet. Do not wash cells before addition of Buffer RZ to avoid increased chance of mRNA degradation. Samples from some yeast may need to be homogenized by using a power homogenizer.

c) If samples have a high content of fat, protein, polysaccharides, or extracellular material such as muscle, fat tissue, and tuberous parts of plants an additional step may be needed. After homogenization, centrifuge the homogenate at 12,000 x g for 10 minutes at 2-8°C to remove the insoluble material (extracellular membranes, polysaccharides, and high molecular mass DNA). The supernatant contains RNA and protein. If the sample has high fat content, there will be a layer of fatty material on the surface of the aqueous phase that should be removed. Transfer the clear supernatant to a fresh tube and proceed with step 2.

d) Some bacterial cells may require a homogenizer.

e) After the cells have been homogenized or lysed in TRI Reagent, samples can be stored at –70°C for up to 1 month.

2. Phase Separation: To ensure complete dissociation of nucleoprotein complexes, allow samples to stand for 5 minutes at room temperature. Add 0.2mL of chloroform per mL of TRI Reagent used. Cover the sample tightly, shake vigorously for 15 seconds, and allow to stand for 2–15 minutes at room temperature. Centrifuge the resulting mixture at 12,000 x g for 15 minutes at 2-8°C. Centrifugation separates the mixture into 3 phases: a red organic phase (containing protein), an interphase (containing DNA), and a colorless upper aqueous phase (containing RNA).

RNA Isolation

1. Transfer the aqueous phase to a fresh tube and add 0.5mL of isopropanol per mL of TRI Reagent (Sample Preparation, step 1) and mix. Allow the sample to stand for 5–10 minutes at room temperature. Centrifuge at 12,000 x g for 10 minutes at 2-8°C. The RNA precipitate will form a pellet on the side and bottom of the tube.
2. Remove the supernatant and wash the RNA pellet by adding minimum of 1mL of 75% ethanol per 1mL of TRI Reagent (Sample Preparation, step 1). Vortex the sample and then centrifuge at 7,500 x g for 5 minutes at 2-8 °C.

Notes:

a) If the RNA pellets float, perform the wash in 75% ethanol at 12,000 x g.
b) Samples can be stored in ethanol at 2-8°C for at least 1 week and up to 1 year at –20°C.

3. Briefly dry the RNA pellet for 5–10 minutes by airdrying or under a vacuum. Do not let the RNA pellet dry completely, as this will greatly decrease its solubility. Do not dry the RNA pellet by centrifugation under vacuum. Add an appropriate volume of formamide, water, or a 0.5% SDS solution to the RNA pellet. To facilitate dissolution, mix by repeated pipetting with a micropipette at 55–60°C for 10–15 minutes.

Notes:

a) Final preparation of RNA is free of DNA and proteins. It should have a A260/A280 ratio of ≥1.7.
b) Typical yields from tissues (mg RNA/mg tissue): liver, spleen, 6-10µg; kidney, 3-4µg; skeletal muscle, brain, 1-1.5µg; placenta, 1-4 µg.
c) Typical yields from cultured cells (mg RNA/10^6 cells): epithelial cells, 8-15µg; fibroblasts, 5-7µg.
d) Ethidium bromide staining of RNA in agarose gels visualizes two predominant bands of small (2kb) and large (5kb) ribosomal RNA, low molecular mass (0.1–0.3kb) RNA, and discrete bands of high molecular mass (7–15kb) RNA.

DNA Isolation

1. Carefully remove the remaining aqueous phase overlaying the interphase and discard. To precipitate the DNA from the interphase and organic phase, add 0.3mL of 100% ethanol per 1mL of TRI Reagent (Sample Preparation, step 1). Mix by inversion and allow to stand for 2–3 minutes at room temperature. Centrifuge at 2,000 x g for 5 minutes at 2-8°C.

   Note: Removal of the remaining aqueous phase before DNA precipitation is a critical step for the quality of the isolated DNA.

2. Remove the supernatant and save at 2-8°C for protein isolation. Wash the DNA pellet twice in 0.1M Trisodium citrate, 10% ethanol solution. Use 1mL of wash solution for every 1mL of TRI Reagent (Sample Preparation, step 1). During each wash, allow the DNA pellet to stand (with occasional mixing) for at least 30 minutes. Centrifuge at 2,000 x g for 5 minutes at 2-8°C. Resuspend the DNA pellet in 75% ethanol (1.5–2mL for each mL TRI Reagent) and allow to stand for 10–20 minutes at room temperature.

   Notes:

   a) Important: Do not to reduce the time samples remain in the washing solution. 30 minutes is the absolute minimum time for efficient removal of phenol from the DNA.

b) If pellet contains >200μg of DNA or large amounts of non-DNA material, an additional wash in 0.1M Trisodium citrate, 10% ethanol solution is required.

c) **Samples suspended in 75% ethanol can be stored at 2-8°C for several months**.

3. Dry the DNA pellet for 5–10 minutes under a vacuum and dissolve in 8mM NaOH with repeated slow pipetting with a micropipette. Add sufficient 8mM NaOH for a final DNA concentration of 0.2–0.3mg/mL (typically 0.3–0.6mL to the DNA isolated from 50–70mg of tissue or 10^7 cells). This mild alkaline solution assures complete dissolution of the DNA pellet. Centrifuge at 12,000 x g for 10 minutes to remove any insoluble material and transfer the supernatant to a new tube.

   Notes:

   a) A viscous supernatant indicates the presence of high molecular mass DNA.

   b) The size of the DNA will depend on the force exerted during homogenization. Avoid using a Polytron homogenizer.

   c) Samples dissolved in 8mM NaOH can be stored at 2-8°C overnight. For long term storage, adjust the pH value to between 7 and 8 and supplement with EDTA (final concentration 1mM).

   d) To determine DNA concentration, remove an aliquot, dilute with water, and measure the A_{260}. For double stranded DNA, 1 A_{260} unit/mL = 50 μg/mL.

   e) Typical yields from tissues (μg DNA/mg tissue): liver & kidney 3–4μg; skeletal muscle, brain and placenta 2–3μg.

   f) Typical yields from cultured human, rat, and mouse cells: 5–7μg DNA/10^6 cells.

Protein Isolation

1. Precipitate proteins (see note) from the phenol/ethanol supernatant (DNA Isolation, step 2) with 1.5mL of isopropanol per 1mL of TRI Reagent (Sample Preparation, step 1). Allow samples to stand for at least 10 minutes at room temperature. Centrifuge at 12,000 x g for 10 minutes at 2-8 °C.

   Note: For some samples, the protein pellet may be difficult to dissolve in 1% SDS (step 3). Use this alternate procedure to correct the problem:

   a) Dialyze the phenol-ethanol supernatant against 3 changes of 0.1% SDS at 2-8 °C.

   b) Centrifuge the dialysate at 10,000 x g for 10 minutes at 2-8 °C.

   c) The clear supernatant contains protein that is suitable for use in Western blotting procedures.

2. Discard supernatant and wash pellet 3 times in 0.3M guanidine hydrochloride or 95% ethanol solution, using 2mL per 1mL of TRI Reagent (Sample Preparation, step 1). During each wash, store samples in wash solution for 20 minutes at room temperature. Centrifuge at 7,500 x g for 5 minutes at 2-8°C. After the 3 washes, add 2mL of 100% ethanol and vortex the protein pellet. Allow to stand for 20 minutes at room temperature. Centrifuge at 7,500 x g for 5 minutes at 2-8°C.
   Note: Protein samples suspended in 0.3M guanidine hydrochloride or 95% ethanol solution or 100% ethanol can be stored for 1 month at 2-8°C or 1 year at –20°C.
3. Dry protein pellet under a vacuum for 5–10 minutes. Dissolve pellet in 1% SDS aided by working the plunger of micropipette with tip in the solution. Remove any insoluble material by centrifugation at 10,000 x g for 10 minutes at 2-8°C. Transfer supernatant to a new tube. The protein should be used immediately for Western blotting or stored at –20°C.

Troubleshooting Guide

1. RNA Isolation:

A. Low yield may be due to:
- incomplete homogenization or lysis of samples.
- the final RNA pellet may not have been completely dissolved.

B. If the A_{260}/A_{280} ratio is <1.65:
- the amount of sample used for homogenization may have been too small.
- samples may not have been allowed to stand at room temperature for 5 minutes after homogenization.
- there may have been contamination of the aqueous phase with the phenol phase.
- the final RNA pellet may not have been completely dissolved.

C. If there is degradation of the RNA:
- the tissues may not have been immediately processed or frozen after removing from the animal.
- the samples used for isolation or the isolated RNA preparations may have been stored at –20°C instead of –70°C as specified in the procedure.
- cells may have been dispersed by trypsin digestion.
- aqueous solutions or tubes used for procedure may not have been RNase-free.
- formaldehyde used for the agarose gel electrophoresis may have had a pH value <3.5.

D. If there is DNA contamination:
- the volume of reagent used for the sample homogenization may have been too small.

- samples used for the isolation may have contained organic solvents (ethanol, DMSO), strong buffers or alkaline solution.

2. DNA Isolation:

A. Low yield may be due to:
- incomplete homogenization or lysis of samples.
- the final DNA pellet may not have been completely dissolved.

B. If the A_{260}/A_{280} ratio is <1.70:
- phenol may not have been sufficiently removed from the DNA preparation. Try one more wash of the DNA pellet with the 0.1M trisodium citrate, 10% ethanol solution.

C. If there is degradation of the DNA:
- the tissues may not have been immediately processed or frozen after removing from the animal.
- the samples used for isolation may have been stored at –20 °C instead of –70 °C as specified in the procedure.
- samples may have been homogenized with a Polytron or other high- speed homogenizer.

D. If there is RNA contamination:
- there may have been too much aqueous phase remaining with the organic phase and interphase.
- the DNA pellet may not have been washed sufficiently with 0.1M trisodium citrate, 10% ethanol solution.

3. Protein Isolation:

A. Low yield may be due to:
- incomplete homogenization or lysis of samples.
- the final protein pellet may not have been completely dissolved.

B. If there is degradation of the protein:
- the tissues may not have been immediately processed or frozen after removing from the animal.

C. If PAGE shows band deformation:
- protein pellet may not have been washed sufficiently.

Appendix

Isolated RNA is to be used in RT-PCR

1. Modifying the procedure by performing the additional centrifugation step in the initial Sample Preparation, step 1B, note c further minimizes the possibility of DNA contamination in the RNA extracted by TRI Reagent LS.
2. A more complete evaporation of ethanol is required when RNA samples are to be used in RT-PCR. This is especially critical for small volume samples (5–20µL), which may contain a relatively high level of ethanol if not adequately dried.

References

1. Chomczynski P. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques. 1993 Sep;15(3):532-4, 536-7.
2. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987 Apr;162(1):156-9

Storage

Store the product at room temperature.
For long-term storage, keep at 4°C for 1 year.