Ver. 24111

ODP419

Total RNA Teaching Kit

For extraction of total RNA from tissue and blood samples

Origin

ISO 13485:2016 ISO 9001:2015

Total RNA Teaching Kit

Kit Contents

Contents	10 Preps
TRI Reagent	10mL
RNase-free water	2mL
Isopropanol	6mL
5X RNA Loading Buffer	30µL
10X MOPS Electrophoresis Buffer	75mL
Formaldehyde 37%	30mL
Agarose	7.2g
Collection Tubes, Polypropylene (1.5mL)	20 Nos.

Introduction

Total RNA Extraction Teaching Kit is designed for rapid extraction and purification of total RNA for Northern analysis, Poly A+ RNA selection, Primer extension, RNase and S1 nuclease protection assays, RT-PCR, Differential display, Expression-array and Expression-chip analysis. Total RNA is extracted using TRI reagent. This is one of the most effective method for isolating total RNA and can be completed in only 1 hour starting with fresh tissue and cells.

Principle

Total RNA Extraction Teaching Kit is designed for rapid purification of RNA from different samples using TRI reagent. This product which is a mixture of guanidine thiocyanate and phenol in a mono-phase solution which effectively dissolves RNA. 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. RNA is precipitated using isopropyl alcohol. Impurities are removed using ethanol and pure RNA is resuspended in RNase-Free Water. 1mL of TRI Reagent is sufficient to isolate RNA from 50-100mg of tissue. This advanced RNA isolation procedure is an improvement to the single-step RNA isolation using phenol and guanidine isothiocyanate developed by Chomczynski and Sacchi.

Duration of Experiment

a) Protocol: 1 hour
b) Agarose Gel Electrophoresis: 1 hour

Materials Required But Not Provided

Glasswares: Conical flask, Measuring cylinder, Beaker
Reagents: Ethidium bromide (10mg/mL), Ethanol, Chloroform, Diethyl pyrocarbonate, RNase-free water for gel electrophoresis, Isopropanol, RNase-free water of 0.5% SDS.
Other requirements: Electrophoresis apparatus, UV Transilluminator, Heating block or Water Bath, Vortex Mixer, Tabletop Micro centrifuge (with rotor for 2mL tubes), Micropipettes, Tips with aerosol barrier, Adhesive tape

Important Instructions

1. Read the entire procedure carefully before starting the experiment.
2. Thoroughly mix the reagents. Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.
3. Ensure the use of only clean & dry DNase, RNase free eppendorf tubes and tips for the procedure.
4. 75% Ethanol for one experiment: Add 0.75mL of ethanol (96-100%) to 0.25mL of RNase-free water.
5. Set the centrifuge at 4°C before starting the experiment.

Precautions to be taken while handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.
2. Collection tubes, tips, pipettes, electrophoresis unit etc to be used for the experiment must be UV treated for 15-20 minutes.
3. Use sterile, disposable plasticware and micropipettes reserved for RNA work to prevent cross- contamination with RNases from shared equipments.
4. Non-disposable plasticwares should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH, 1mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.
5. Glassware used for RNA work should be cleaned with detergent, thoroughly rinsed, and oven baked at 240°C for at least four hours before use. Alternatively, glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.
6. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
7. Solutions (water and other solutions) should be treated with 0.1% DEPC.

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)
Peripheral Blood Mononuclear Cells(PBMC)	Isolated from 5mL whole blood by density–gradient centrifugation

Note: 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, therewill be a layer of fatty material on the surface of the aqueous phasethat should be removed. Transfer the clear supernatant to a fresh tube and proceed with step 2.

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).
   Note: The chloroform used for phase separation should not contain isoamyl alcohol and other additives.
3. 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.
4. 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) **Be careful while discarding / decanting the supernatant as the pellet might also get discarded along with the supernatant.**
   b) **If the RNA pellets float, perform the wash in 75% ethanol at 12,000 x g.**
   c) **Samples can be stored in ethanol at 2-8°C for at least 1 week and upto 1 year at –20°C.**
5. 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) **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.**
b) **Final preparation of RNA is free of DNA and proteins. It should have a A_{260}/A_{280} ratio of ≥ 1.7.**
c) **Typical yields from tissues (mg RNA/mg tissue): liver, spleen, 6-10µg; kidney, 3-4µg; skeletal muscle, brain, 1-1.5µg; placenta, 1-4µg.**
d) **Typical yields from cultured cells (mg RNA/10^6 cells): epithelial cells, 8-15µg; fibroblasts, 5-7µg.**
e) **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.**

Agarose Gel Electrophoresis

Preparation of 1X MOPS Electrophoresis Buffer: To prepare 500mL of 1X MOPS Electrophoresis buffer, add 50mL of 10X MOPS Electrophoresis Buffer to 440mL of RNase-free water and add 10mL of Formaldehyde (37%). Mix well before use.

Preparation of agarose gel: To prepare 50mL of 1.2% agarose solution, mix 5mL of 10X MOPS Electrophoresis Buffer with 45mL of autoclaved deionized water in a glass beaker or flask. To this add 0.6g of agarose. Heat the mixture in a microwave, burner or hot plate by swirling the glass beaker/ flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow solution to cool to about 55-60°C. Add 0.9mL of 37% Formaldehyde and mix well. Immediately add 0.5µL Ethidium Bromide, mix well and pour the gel solution into the gel tray sealed on both sides with adhesive tape. Allow the gel to solidify for about 30 minutes at room temperature (15-25°C).
Note: Before running the gel, equilibrate it in 1X MOPS Electrophoresis Buffer for at least 30 minutes.

Loading of the RNA samples: To prepare sample for electrophoresis, add 2µL of 5X RNA Loading Buffer per 10µL of RNA sample. Load onto the equilibrated MOPS gel.

Electrophoresis: Connect power cord to the electrophoretic power supply according to the conventions: Red- Anode and Black- Cathode. Electrophorese at 100V and 70mA until dye markers have migrated an appropriate distance, depending on the size of RNA to be visualized.

Quantitation of RNA:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use RNase-free Water to dilute the samples and to calibrate the spectrophotometer, measure the absorbance at 260nm, 280nm, and 320nm using a quartz microcuvette. Absorbance readings at 260nm should fall between 0.1 and 1.0. The 320nm absorbance is used to correct for background absorbance. Purity is determined by calculating the ratio of absorbance at 260nm to absorbance at 280nm. The concentration of RNA is calculated by the following formula:

Concentration of RNA sample (µg/mL) = 40 x A_{260} x dilution factor.

Troubleshooting Guide

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 temperaturefor 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 mayhave 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.

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, further minimizes the possibility of DNA contamination in the RNA extracted by TRI Reagent.
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

Total RNA Extraction Teaching Kit can be stored at room temperature (15-25˚C) for up to 1 year from the date of manufacture without showing any reduction in performance. On receipt, store 5X RNA Loading Buffer and TRI Reagent at 2-8˚C. Other reagents can be stored at room temperature (15-25˚C).