TTI

TL-4100
TL-4110
TL-4120

RNA-DNA STAT 60

Two Single Extraction Reagents For The Separate Isolation Of Total RNA/mRNA And DNA From Same Sample

Cat. No. TL-4100 FOR IN VITRO RESEARCH USE TEL-TEST "B" Bulletin #6

Storage: Keep at 4°C, in dark. This product is light sensitive.

INTRODUCTION

The entire procedure for RNA/DNA isolation using RNA-DNA STAT 60 can be completed in less than 90 minutes. These are the most effective methods for isolation of RNA and DNA isolation from the same sample. The recovery of undegraded RNA and DNA is 30-150% greater than with any other method of RNA/DNA isolation.

APPLICATION

The total RNA/DNA isolated by RNA-DNA STAT 60 is used for Northern and Southern analysis, dot-blot hybridization, poly A+ selection, invitro translation, RNase protection assay, molecular cloning, and polymerase chain reaction from human, animal, plant without any additional RNase/DNase treatment. Multi-sample processing is a great advantage of using these simple single reagents. Excellent recovery of RNA/DNA permits the use of this product for isolation of RNA/DNA from very small biological samples (biopsies etc.).

REAGENTS SUPPLIED

RNA STAT-60 and DNA STAT-60

Preparation: Ready to use.

Storage: Refrigerate at 2-8°C.

Stability: Refer to expiration date stamped on label.

REAGENTS REQUIRED BUT NOT SUPPLIED

Chloroform(ACS grade), Isopropanol(ACS grade), and Ethanol(ACS grade).

PROTOCOL

RNA/mRNA isolation by the RNA-DNA STAT 60 method includes the following steps:

1. Homogenization RNA STAT-60 (1ml per 50-100mg tissue, or 5-10 x 10^6 cells
2. RNA Extraction 1 vol. of homogenate + 0.2 vol. of chloroform
3. RNA Precipitation 0.5 vol. of isopropanol
4. RNA Wash 75% ethanol

Unless stated otherwise the procedure is carried out at room temperature.

5.1 HOMOGENIZATION:

A. TISSUES:

Homogenized tissue samples in the RNA STAT-60 (1ml/50-100mg tissue) in a glass-Teflon or Polytron homogenizer. Sample volume should not exceed 10% of the volume of the RNA STAT-60 used for homogenization.

B. CELLS:

Cells grown in monolayer are lysed directly in a culture dish by adding the RNA STAT-60™ (1ml/3.5cm petri dish) and passing the cell lysate several times through a pipette. Cells grown in suspension are sedimented then lysed in the RNA STAT-60™ (1ml per 5-10 x 10^6 cells) by repetitive pipetting. Washing cells before addition of the RNA STAT-60™ should be avoided as this increases the possibility of mRNA degradation.

5.2 RNA EXTRACTION:

Following homogenization, store the homogenate for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. next, add 0.2ml of chloroform per 1ml of the RNA STAT-60™ cover the sample tightly, shake vigorously for 15 seconds an let it stay at room temperature for 2-3 minutes. Centrifugate the homogenate at 12,000g (max) for 15 minutes 4°C. Following centrifugation, the homogenate separates into two phases: a lower red phenol chloroform phase and the colorless upper aqueous phase. RNA remains exclusively in the aqueous phase whereas DNA and proteins are in the interphase and organic phase. The volume of the aqueous phase is about 60% of the volume of RNA STAT-60™ used for homogenization.

5.3 RNA PRECIPITATION:

Transfer the aqueous phase to a fresh tube and mix with isopropanol. Add 0.5ml of isopropanol per 1 ml of the RNA STAT-60™ used for homogenization. Store samples at room temperature for 5-10 minutes and centrifuge at 12,000g (max.) for 10 minutes at 4°C. RNA precipitate (often visible before centrifugation) forms a white pellet at the bottom of the tube.

Remove supernatant and wash the RNA pellet once with 75% ethanol by vortexing and subsequent centrifugation at 7,500g (max.) for 5 minutes at 4°C. Add at least 1ml of 75% ethanol per 1ml of the RNA STAT-60™ used for the initial homogenization.

At the end of the procedure, dry the RNA pellet briefly by air-drying or in a vacuum (5-10 min.). It is important not to let the RNA pellet dry completely as it will greatly decrease its solubility. Do not use the Speed-Vac for drying. Dissolve the RNA pellet in water or in 1mM EDTA, pH 7, or 0.5% SDS solution. Vortex or pass the pellet a few times through a pipette tip. An incubation for 10-15 minutes at 55-60°C may be required to dissolve RNA samples. Diethylpyrocarbonate (DEPC) treated RNase-free solutions should be used for solubilization of RNA.

6. EXPECTED YIELD AND PURITY:

Expected yield of total RNA:

a) Tissues (µg/mg tissue): liver, spleen, 7-10 µg; kidney, 3-4 µg; skeletal muscles, brain, 1-1.5µg; placenta 1-4 µg.

b) Cultured cells ($\mu g/10^6$ cells): epithelial cells, 10-15 µg, fibroblasts, 5-7 µg.

The final preparation of total RNA is free of DNA and proteins and has a 260/280 ratio > 1.8.

7. NOTES AND COMMENTS:

1. For isolation of RNA from a small amount of cells or tissue (1-10mg): homogenize samples in 0.8ml of the RNA STAT-60™, transfer the homogenate to the eppendorf tube and follow the isolation protocol with the exception of the RNA precipitation which should be carried out for 30 minutes at 4°C.

2. Following homogenization (before addition of chloroform) samples can be stored at -70°C for at least 2 weeks.

3. An additional precipitation may be necessary to use RNA isolated by the RNA STAT-60™ in enzymatic assays. Following solubilization, precipitate RNA in the presence of 0.2 M NaCl with two volumes of ethanol for 15 minutes at 4°C. The PCR and RNase protection assays do not require this traditional precipitation step.

4. Hand and dust may be the major source of the RNase contamination. Use gloves and keep tubes closed. The use of sterile, disposable polypropylene tubes is recommended throughout the procedure.

The RNA STAT-60™ contains poison (phenol) and irritant (guanidinium thiocyanate). CAN BE FATAL. When working with the RNA STAT-60™ use gloves and eye protection (shield, safety goggles). Do not get on skin or clothing. Avoid breathing vapor. Read also the warning note on the bottle.

PROTOCOL FOR DNA REVERSE EXTRACTION FROM SAMPLE USED FOR RNA EXTRACTION

DNA REVERSE EXTRACTION

Remove aqueous layer containing RNA. Add 800ul of DNA STAT-60 reagent per 1ml of RNAzol, RNAzol B or RNA STAT-60 used for the initial homogenization. Add 0.2ml of chloroform per 1ml of DNA STAT-60, shake vigorously for 15 seconds and let it stay at room temperature for 2-3 minutes. Centrifuge the homogenate at 2000g(min)-12,000g(max) for 15 minutes 4°C. Following centrifugation the homogenate separates into two phases: a lower organic phase and the upper aqueous phase. DNA remains in the aqueous phase whereas RNA and proteins are in the interphase and organic phase.

DNA PRECIPITATION

Transfer the aqueous phase to a fresh tube and mix with isopropanol. Add 0.5ml of isopropanol per 1ml of the DNA STAT- 60 used for homogenization store samples at room temperature for 5-10 minutes and centrifuge at 12,000g(max) for 10 minutes at 4°C. The DNA precipitate forms a small clear to white pellet at the bottom of the tube.

DNA WASH

Remove supernatant and wash the DNA pellet once 75% ethanol by vortexing and subsequent centrifugation at 7,500g (max) for 5 minutes at 4°C. Add at least 1ml of 75% ethanol per 1ml of the DNA STAT-60 used for the initial homogenization.

At the end of the procedure, dry the DNA pellet briefly by air drying or in a vacuum (5-10 min.). Dissolve the DNA pellet in water or in 1mm EDTA, pH 7. Vortex or pass the pellet a few times through a pipette tip. An incubation for 10-15 minutes at 55-60°C may be required to dissolve DNA samples.

SPECIAL HANDLING PRECAUTIONS

The DNA STAT-60™ contains an irritant (guanidinium Salts). Can be fatal. When working with DNA STAT-60 use gloves and eye protection (shield, safety goggles). Do not get on skin or clothing. Avoid breathing vapor. Read warning note on bottle.

REFERENCES

1. Chomczynski, P. and Sacchi, 1987, Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. Biotechniques 8:148-149.

Additional references available upon request.

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