OZYME
Des femmes et des hommes
au service de vos recherches

RNA NOW™

60 MINUTE RNA ISOLATION METHOD

Total RNA and Messenger RNA Enrichment Reagent

1. INTRODUCTION

The isolation of consistent high quality intact RNA from a wide variety and large number of biological samples has proven to be a complicated problem. Progress in methods of RNA isolation has made it possible to replace lengthy and laborious methods of total RNA isolation by a single step methods [3,5].

The RNA NOW "One Step" 60 minute method (US PATENT) provides for the rapid and consistent isolation of high quality intact total RNA and the enrichment of messenger RNA in high yield, that is free of DNA. Advantages over other single RNA reagents include DNA free RNA isolation, which eliminates further sample manipulations and subsequent potential loss of rare species of RNA. The RNA NOW™ single reagent contains a proprietary Phenol /alcohol containing RNA extraction reagent pH adjusted to enrich for messenger RNA. The entire method can be completed in sixty minutes. The isolated total RNA is particularly suitable for northern analysis, solution hybridization, molecular cloning, RT-PCR, and enzymatic assays.

RNA NOW™ FEATURES

- •TOTAL RNA ISOLATION, FREE OF CONTAMINATING DNA WITHIN 60 MINUTES
- •ENRICHES FOR RARE SPECIES OF mRNA
- •HELPS OPTIMIZE, ENHANCE, AND STANDARDIZE RNA AMPLIFICATION REACTIONS
- •NORTHERN BLOT / RT-PCR READY mRNA
- •CONSISTENT 260 / 280 ABSORBANCE RATIOS GREATER THAN 1.9
- •COST - EFFECTIVE METHOD REQUIRING LESS REAGENT / SAMPLE

2. INTENDED USE

The reagent is indicated for the rapid isolation of total RNA and the enrichment of messenger RNAs from tissues and cells of animal, plant, yeast, and bacterial origin. The isolated RNA is suitable for Solid-phase extraction, Northern blot, solution hybridization, and RT-PCR. In addition, the RNA can be used for polyA+ selection, RNase protection assay, and *in vitro* translation. The RNA NOW™ method can also be used for the simultaneous processing of multiple cell or tissue samples, including biopsies and human bodily fluids such as stool, sputum, urine, wound exudate, and blood.

3. REAGENTS INCLUDED:

RNA NOW™ : 100 or 200 ml bottles
PREPARATION: Gently shake reagents before use.
Color: Blue
Storage: Refrigerate at 4º C. Protect from light.
Stability: 18 months. Refer to expiration date on label.
NOTE: A small portion of the extraction reagent may form crystals or settle at bottom of bottle. This is normal and should not affect performance of reagent.

4. ITEMS REQUIRED:

Chloroform (ACS grade), Isopropanol (ACS) grade, Ethanol 100% (ACS) grade, DEPC water, 5cc syringe, 20-Gauge needle.

5. PROTOCOL:

1. CELL LYSIS / HOMOGENIZATION: RNA NOW™
RNA NOW™ REAGENT (1 ml per 10-100 mg tissue, or 5-10x10^6 cells). To isolate RNA from small amounts of tissue (< 20 mg) or a small number of cells (< 5x 10^6 cells), use no less than 1 ml of the reagent.

2. RNA EXTRACTION:
1 vol. homogenate + 0.2 vol. of chloroform

3. RNA PRECIPITATION:
One volume of aqueous phase + one volume of isopropanol.

4. RNA WASH:
One volume of 75% ethanol per volume of the RNA NOW™ used in the initial homogenization (an optional second wash may be required for best results). Unless stated otherwise carry out protocol at room temperature.

5.1 HOMOGENIZATION:

A. TISSUES: Homogenize tissue samples in the RNA NOW™ reagent (1 ml /20-100 mg tissue) using a mechanical homogenizer, glass Teflon homogenizer, or a disposable all glass micro-homogenizer. Do not use less than 1 ml of the reagent.

B. CELL SUSPENSIONS: To isolate RNA from cells grown in suspension, sediment cells and lyse them by the adding 1 ml of the RNA NOW™ reagent per 5-10 x 10^6 cells.

C. MONOLAYER CULTURE: Cells grown in tissue cultureware are lysed directly in the cultureware by the addition of the RNA NOW™ REAGENT [1 ml per 3.5 cm petri dish or 10 cm^2] and passing the cell lysate several times through a pipette. Transfer he cell lysate immediately into a sterile microfuge tube. Note: Do not leave the reagent in contact with plastic plates for more than a few minutes.

D. CELLS GROWN IN SUSPENSION: Cells grown in suspension are sedimented, cell culture medium is aspirated and the cell pellets are lysed in the the RNA NOW™ (1 ml per 5-10 x 10^6 cells) by repetitive pipetting. Note: Do not wash cells after pelleting as this increases the chances of RNA degradation.

E. LIQUID MATRICES: To isolate RNA from liquid matrices including blood, stool, sputum, urine, wound exudate and viral cultures, homogenize samples in 10 ml of the cell lysis reagent per 1 ml of the liquid sample. Solubilize the RNA by repetitive pipetting.

5.2 RNA EXTRACTION:

Following homogenization, add 0.2 ml of chloroform per 1ml of homogenate and shake sample by hand for 20 seconds. DO NOT VORTEX. Place sample on ice for 5 minutes. Centrifuge samples at 12,000 x g (4°C) for 10 minutes. After centrifuging, the homogenate layers into two phases; the lower, blue phenol-chloroform phase and the clear, upper, aqueous phase. The RNA remains in the clear, upper, aqueous phase.

5.3 RNA PRECIPITATION:

Carefully (without getting too close to the inter-phase) transfer the aqueous phase to a new tube, and add an equal volume of isopropanol. Mix well by hand and store samples on ice for 5 minutes. Centrifuge at 4°C at >10,000 x g for 10 minutes to pellet precipitated RNAs.

5.4 RNA WASH:

Decant the supernatant and wash the RNA pellet 1-2 times with excess (use at least 1ml of 75% ethanol per 1ml of the RNA NOW™ reagent used in the initial homogenization step) 75% ethanol. Vortex, and centrifuge at 2,000 –5000 x g for 5 minutes at 4°C. DO NOT CENTRIFUGE THE SAMPLE AT MORE THAN 7,500 x g. Following the ethanol wash(s), air dry the RNA pellet for 10-15 minutes or, alternatively, dry the RNA briefly under vacuum. DO NOT USE A SPEED□VAC. Finally, dissolve the RNA pellet in RNase-free water, or the appropriate buffer for subsequent procedures.

NOTE: An additional incubation step of heating the RNA sample for 10 minutes at 60°C may be required to completely solubilize over□dried RNA pellets. Over-dried RNA pellets are difficult to solubilize and can result in lower than expected yields and lower 260 / 280 ABS ratios.

6.0 EXPECTED YIELD:

a) Tissues (µg/mg tissue): Mouse (Liver and Spleen, 6-9 µg; Kidney, 2-5 µg; Heart, 1 µg and Brain, 0.5- 2 µg) b) Cultured cells (µg / 1x106 cells): Rat Lymphocytes, 8-15 µg; Epithelial cells, 12-14µg.

7.0 NOTES AND COMMENTS:

7.1 The RNA homogenates (before adding chloroform) may be conveniently stored at between -20 and -80°C for up to one month.

7.2 The procedure may be conveniently interrupted at step 5.3, after adding isopropanol and before centrifuging. Store samples at between -20 and -80°C overnight. This optional step is recommended when isolating RNA from small samples or when trying to optimize yield.

7.3 An additional precipitation step may be required when isolating RNA for enzymatic assays. Following RNA hydration, re-precipitate the RNA with two volumes of ethanol containing 0.2M NaCl for 15 minutes at 4°C. The RT-PCR and RNase protection assays do not require this re-precipitation step.

7.4 DNA/ protein contamination may result if the entire aqueous phase is removed. To avoid DNA/protein contamination, exercise care while decanting the aqueous phase. Never try removing more than 90% of the aqueous phase.

8.0 SPECIAL HANDLING PRECAUTIONS:

The RNA NOW™ reagent (UN2821) contains a class B (6.1) Poison (phenol) and chaotropic irritants. The reagent can be fatal. When handling the reagents wear gloves and eye protection. Do not get on skin or clothing. Avoid breathing vapor. In case of contact, immediately flush affected area with copious amounts of water for at least 15 minutes and seek immediate medical attention.

9.0 REFERENCES:

1. Chirgwin, J.M., et al. 1979. Biochem. 18 (24): 5294 - 5299.
2. Maniatis, T., Fritsch, E.F., and Sambrook, J. 1982. "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. pp 7.19-7.22.
3. Chomczynski, P., and Sacchi, N. 1987. Anal. Biochem 162: 156-159.
4. Puissant, C. and Houdebine, L. 1991. Biotechniques 8: 148 -149.
5. Biogentex. Methods and compositions for isolating nucleic acids 1997. US PATENT 5,637,687 / CIP
6. Biogentex. Product and Process for Isolating DNA with a novel phenol free solvent system. US PATENT 5,637,687

RNA NOW™ is a trademark of Biogentex and the subject of US Patent 5,637,687 CIP / PCR is the subject of patents granted to Cetus Corporation.

GHS SPECIAL HANDLING PRECAUTIONS / PICTOGRAMS

WARNING **DANGER** **POISON**

RNA NOW™
Toxi / Irr (UN 2821, 6.1, II)
Contains Phenol and Guanidine compounds.
Keep out of the reach of children.
Read label before use. Do not taste or swallow.
Do not take internally. Wash thoroughly after handling. Use only in adequate ventilation.
Do not eat, drink or use tobacco when using this product. Wear protective gloves and eye/face protection. Wash hands thoroughly after handling.
Keep container tightly closed. Do not breath fume /gas/mist/vapor/spray. See MSDS for further details regarding safe use of this product.

The product is toxic by ingestion, inhalation, and skin contact. May cause liver or kidney damage through prolonged or repeated exposure.
FIRST AID: If contact with skin: Remove contaminated clothing. Flush exposed skin or eyes with large amounts of water for at least 15 minutes.
If inhaled: Remove to fresh air and keep at rest in a position comfortable for breathing.
If swallowed: Immediately call a doctor.

Service client - commande : commande@ozyme.fr
Service technique :
Réactifs : 01 34 60 60 24 - tech@ozyme.fr
Instrumentation : 01 30 85 92 88 - instrum@ozyme.fr