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Isolation of RNA by the Guanidine Thiocyanate (GTC) Method in Thermo Scientific Swinging Bucket Rotors

Linda D. Santomenna, Central Research and Development Department, E.I. Du Pont de Nemours and Company, Wilmington DE 19898
Stephanie R. Noles, PhD, Thermo Fisher Scientific, Laboratory Equipment Division, Associate Product Manager

Key Words

- RNA Isolation
- Guanidine Thiocyanate (GTC)
- Cesium Chloride Gradient
- Ultracentrifugation
- Swinging Bucket Rotor

Introduction

Infection of human cells by viruses is a complex interaction that can result in cell death and production of progeny virus or, alternatively, in latency. Human cytomegalovirus (HCMV) replicates in permissive human diploid fibroblasts and increases the abundance of certain cellular transcripts, including ornithine decarboxylase, thymidine kinase, heat-shock protein 70 and brain creatine kinase[1]. RNA isolation to detect these transcripts is important to scientific research of viral infection.

A classic method for the isolation of RNA is the guanidine thiocyanate (GTC) method[2] which involves pelleting through a cesium chloride (CsCl) cushion in a swinging bucket rotor at 125,000 – 150,000 x g. This method permits the isolation of intact RNA that has been separated from cellular and viral DNA, proteins and lipid components.

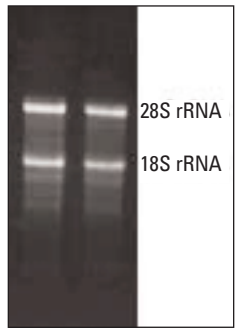
Thermo Scientific swinging bucket rotors TH-641 and AH-650 are used to accommodate the variable RNA yields due to different cell types.

Procedure

Wash infected cells twice with phosphate buffered saline (PBS), then apply GTC buffer (1.5 mL/10^7 cells) to the monolayer. Gently rock the flask to ensure even distribution, and allow the GTC to set on the cells for 10 minutes. Collect the GTC by standing the flask on end, and transfer the suspension to a polypropylene tube. Shear the DNA in the GTC suspension by pressing this material progressively through smaller needles, beginning with an 18 gauge needle and finishing with a 26 gauge needle. As a result, the suspension should no longer be viscous. Pretreat polyallomer (PA) centrifugation tubes by rinsing the inside of them with GTC. Fill the bottom 1/3 of the tube with 5.7 M CsCl in a sodium acetate buffer (pH 5.0). Gently load the GTC suspension onto the CsCl cushion. Spin large samples (>5 x 10^7 cells or volumes up to 13.2 mL) in a TH-641 rotor at 31,000 rpm (164,450 x g) using PA thin-walled tubes (catalog no. 03699)

Spin smaller samples (volumes up to 5mL) in the AH-650 rotor at 36,000 rpm (153,450 x g) using PA tubes (catalog no. 03127). Either gradient should be run for 18-22 hours at 20°C in a Thermo Scientifc Sorvall® WX ultracentrifuge. A gelatinous RNA pellet will form at the bottom of the tube and the DNA will form a band near the interface of the CsCl and the GTC. Mark the position of the DNA band on the tube, then remove all but 1 mL, of the supernatant, discarding the DNA. Cut the tube below the DNA band previously marked on the tube, and pour off the remaining supernatant. Resuspend the RNA pellet in 300 µL of diethylpyrocarbonate (DEPC)-treated water then heat for 10 minutes at 65°C. Place the RNA in a sterile microtube. Wash the centrifuge tube with another 100 µL of DEPC-treated water and pool this with the other RNA. Precipitate the RNA overnight in 2 volumes of ethanol and 1/10th volume of 3 M sodium acetate (pH 5.4). Pellet the RNA, then resuspend in DEPC-treated water. This RNA can be stored for several months at -70°C. Check the integrity of the RNA by electrophoretic separation of 1-2 µg of total RNA in an agarose/urea or an agarose/formaldehyde gel.

Results and discussion



After electrophoresis, the 28S (upper bands) and the 18S (lower bands) ribosomal RNA species can be visualized by staining the gel with ethidium bromide. This RNA is intact and quite suitable for Northern blotting and subsequent hybridization. Most importantly, this method allows the detection of RNA transcripts in low abundance.

The yield of RNA varies between cell types and is governed by several factors including genetics, cell size and cell density. The flexibility in rotor capacity allows researchers to work with a variety of cell types and consistently achieve reproducible results.

This procedure, in combination with Thermo Scientific rotors and the WX ultracentrifuge, permits RNA studies that help scientists better understand the interaction between viruses and their host cells.

References

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North America:
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+1 800 553 0039

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Belgium
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Germany national toll free
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