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Isolation of RNA Using Thermo Scientific Rotors and Sorvall Discovery SE Microultracentrifuges

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KEY WORDS

- RNA Isolation
- Cesium Chloride
- Guanidine Thiocyanate
- S150-AT Fixed-Angle Rotor
- S55-S Swinging Bucket Rotor
- Microultracentrifuge
- Microcentrifuge

Introduction

Many protocols exist for the extraction and purification of total RNA from cells or tissue. The key to obtaining high quality RNA is the rapid lysis of the cells, the thorough denaturation of endogenous nucleases, and ultimately, the efficient removal of these nucleases. Early methods of RNA purification relied on the use of guanidinium thiocyanate (GTC) for the rapid denaturation of cellular proteins and extraction of RNA by ultracentrifugation through a cesium chloride (CsCl) cushion or a single step procedure that uses a mixture of GTC and phenol-chloroform. Recent techniques and commercially available kits have been developed around these methods that allow for a more rapid process for RNA isolation. Using microcentrifuges, these methods have been found to decrease the time for RNA isolation from a few hours to less than an hour. This brief will provide protocols for the traditional methods of RNA isolation using fixed-angle and swinging bucket rotors in a Thermo Scientific Sorvall Discovery SE microultracentrifuge, and will also identify commercially available kits that are compatible with Thermo Scientific microcentrifuges.

Procedures

PROTOCOL 1: Rapid Isolation of RNA from Cultured Cells Using the Thermo Scientific S55-S Swinging Bucket Rotor and Sorvall® Discovery™ SE Microultracentrifuge[5]

This report describes the use of GTC and centrifugation through a CsCl cusion with a S55-S swinging bucket rotor to isolate RNA from cells grown in culture. This procedure has been developed for working with small samples and offers a high yield (>90%) of RNA in a short period of time (approximately 3 hours).

Lysis

1. Remove media from cell culture plate.
2. Add 600-1000 µL of GTC solution (4.0 M guanidine thiocyanate, 0.03 M NaOAc, 1% β-mercaptoethanol) to each well of the culture plate to lyse.
3. Lift cells with a cell scraper and homogenize the slurry by aspirating 4-5 times through a 22-guage needle.

Isolation

1. Pre-load 2.2 mL polyallomer tubes (Cat. No. 45240) with 550 µL of 5.7 M CsCl and mark solution level with a waterproof pen (Figure 1).
2. Layer samples onto the CsCl cusion and fill tubes to within 3 mm of the top of the tube with GTC solution.
3. Place tubes in an S55-S swinging bucket rotor and centrifuge in a Sorvall Discovery SE microultra-centrifuge at 55,000 rpm (258,826 x g) for 3.0 – 3.2 hours at 22 °C. (Note: Non-precipitating solutions up to 1.7 g/mL can be run in the S55-S rotor without a reduction in rotor speed. Consult rotor manual for instructions on rotor de-ration and how to reduce the chance of CsCl precipitation).
4. Following centrifugation, use a sterile Pasteur pipette to remove the top layer of solution down to the interface reference mark (Figure 2).

Thermo Scientific Sorvall Discovery SE Microultracentrifuge

Thermo Scientific S55-S Swinging Bucket Rotor

5. Using a second sterile pipette, remove remaining solution down to approximately 250 µL.
6. Carefully, cut off the top of each tube just below the reference mark, pour off the remaining solution, and blot the tubes dry on a clean paper towel.

Recovery

1. Rinse RNA pellet x 2 with 75 µL of 70% isopropanol/0.3 M sodium acetate and blot the tubes dry on a clean paper towel.
2. Add 100 µL of DEPC-treated water to each tube and freeze (-70 °C) for a minimum of 45-60 minutes. (Note: DEPC is a suspected carcinogen; follow manufacturers recommended handling procedures).

3. Resuspend RNA by pipetting up and down several times.
4. Transfer solution to a 1.5 mL microcentrifuge tube.
5. Wash the ultracentrifuge tube with a second 100 µL aliquot of 0.25 volumes of 7.5 M ammonium acetate and 2.6 volumes of 100% ethanol.
6. Collect precipitated RNA by centrifugation at >10,000 x g for 20 minutes at 4 °C in a Thermo Scientific Heraeus Pico or Fresco refrigerated microcentrifuge.
7. Wash the pellet with cold 70% ethanol and vacuum dry.
8. Resuspend RNA pellet in a small volume of DEPC-treated water.

PROTOCOL 2: Separation of Total RNAs from a Homogenated Cell Line[4] with the S150-AT Fixed-Angle Rotor in Less than 2 Hours

This procedure describes the separation of total RNAs from a homogenate cell line using the Thermo Scientific S150-AT fixed-angle rotor and the Sorvall Discovery M150 SE microultra-centrifuge, which has a maximum speed of 150,000 rpm. This procedure can separate total RNAs in only 1.75 hours.

1. Pellet cells (5-10 x 10^6 cells/tube).
2. Add 1 – 1.5 mL of 4M GTC and vortex to lyse cells.
3. Layer the samples onto 0.8 mL 5.3 M CsCl solution that has been pre-loaded in a 2 mL polyallomer Re-Seal™ tube (Cat. No. 45246).
4. Place tubes in the S150-AT fixed-angle rotor and centrifuge in a Sorvall Discovery M150 SE microultracentrifuge at 150,000 rpm (899,740 x g) for 1.75 hours at 20 °C. Use acceleration setting of 5 and deceleration setting of 7.
5. Dissolve RNA pellet into buffer and precipitate by ethanol. (Note: Separation of total RNAs may not be completed due to crystallization during centrifugation when 5.7M CsCl is used.)

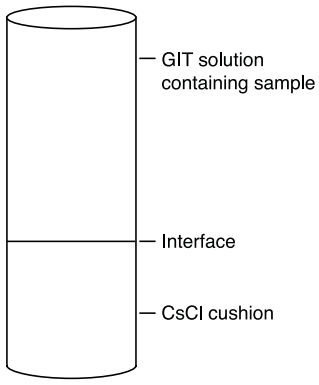

Figure 1. Polyallomer tube containing the sample (GIT solution) on top of the CsCl cushion.

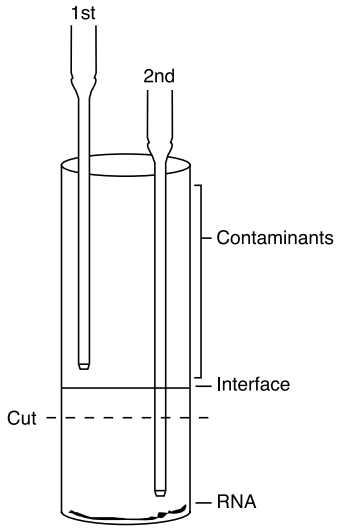

Figure 2. Withdrawal of the supernatant following centrifugation. The figure illustrates the depth of the pipette tips and the cut position relative to the reference mark placed on the tube delineating the interface between the sample and the CsCl cushion.

Conclusion

The isolation and purification of RNA can be accomplished using multiple methods. This brief describes traditional approaches for RNA purification using ultracentrifugation with swinging bucket and fixed-angle rotors. A swinging bucket rotor is often used because the RNA is collected at the bottom of the tube. Although centrifugation in a vertical or fixed-angle rotor requires less time for RNA purification, it may result in materials collecting or coming in contact with the sides of the tube, thus potentially contaminating the RNA. Alternatively, commercially available kits (ie, Promega® Ambion®, Qiagen™) have been developed that are compatible with Thermo Scientific microcentrifuges and may offer enhanced time savings, while increasing RNA yield and purity.

References

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