Application Note:
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Rapid Isolation of RNA by Guanidinium Thiocyanate/Cesium Chloride Gradient Using Thermo Scientific Fiberlite Ultracentrifuge Rotors

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KEY WORDS

- **Cesium Chloride (CsCl) Gradient**
- **RNA Isolation**
- **Guanidinium Thiocyante (GITC)**

Introduction

The guanidinium thiocyanate method for isolation of ribonucleic acid (RNA) is used frequently when undegraded mRNA is desired[1]. The RNA is pelleted through a high density cesium chloride (CsCl) solution to free it of DNA and polysaccharide contamination[1,2]. Customarily, this step is performed in a ultracentrifuge swinging bucket rotor for 12-22 hours. This report describes methods using Thermo Scientific Fiberlite carbon fiber fixed angle ultracentrifuge rotors to reduce run times to 4.5-5 hours allowing RNA isolation in a single day.

Procedures

The described RNA isolation protocol is derived from Sures and Crippa with minor modifications to prepare poly(A)+ RNA from cockroach (*Periplaneta americana*) heads[3,4].

1. Harvest 25 g of tissue, freeze in liquid nitrogen (N_2) and grind to a powder under liquid N_2 with a mortar and pestle.
2. Resuspend powder in 150 mL of buffer containing 4.5 M guanidinium thiocyanate /50 mM EDTA (pH 8.0), 25 mM sodium citrate (pH 7.0), 0.1 M 2-mercaptoethanol, 2% lauroylsarcosine.
3. Homogenize suspension on a medium setting for 3-5 minutes at 4° C.
4. Remove insoluble material by centrifuging the suspension in a Fiberlite® F13-14x50cy carbon fiber rotor at 8,000 xg for 10 minutes at 4° C.
5. Add CsCl to the supernatant at a concentration of 0.2 g/ml.
6. Layer the sample solution over 10 mL of 5.7 M CsCl containing 50 mM EDTA (pH 7.3). If using a polyallomer tube, ensure the tube is completely full and the 5.7 M CsCl cushion should occupy one-third of the total tube volume. If necessary top off tube with 0.2 g/ml CsCl buffered solution.
7. Centrifuge sample solution in Fiberlite F50L-8x39 rotor at 50,000 rpm (~266,000 xg) for 5 hours at 20°C.

NOTE: Recommended conditions for other rotors are provided in Table 1.

8. After centrifugation, remove RNA pellet and dissolve in 2.0 mL of 10 mM Tris-HCl (pH 7.4), 2 mM EDTA.
9. To further purify, extract RNA 3 times with 2 volumes of phenol/ chloroform (1:1) followed by 1 extraction with chloroform (Total RNA yield = 3.2 mg).
10. Isolate poly(A)+ RNA by 2 cycles of oligo(dT)-cellulose chromatography[5] (Final poly(A)+ yield = 42 µg).

Figure 1: Thermo Scientific Fiberlite F65L-6x13.5 rotor

Conclusion

To confirm the integrity and biological activity of the RNA, the mRNA preparation can be translated *in vitro*. Using 200 ng of mRNA in a rabbit reticulocyte translation system, an 80 fold stimulation over background can be expected as determined by 35S-methionine incorporation into protein. The stimulation level will be comparable to that of the globin control. The translation products can be separated on a 12% polyacrylamide gel[6]. A wide range of protein sizes will be synthesized including some larger than 100kDa.

The mRNA can also be used in the construction of a cDNA library according to the first strand reaction conditions described by Gubler and Hoffman[7]. In previous studies, the yields were approximately 20% (µg cDNA/ µg mRNA) as judged by ^{32}P-labeled dCTP incorporation[7]. The cDNA products from this study had a wide size distribution including some

Figure 2: Thermo Scientific Fiberlite F50L-8x39 rotor

that appeared to be full-length copies of their corresponding mRNAs.

Using suggested protocols (Table 1), this method of RNA preparation in a Fiberlite carbon fiber fixed angle rotor, will result in a significant run time reduction. In order to maximize yield and stability use acceleration and deceleration rate of 8/8 (the second fastest setting) in Thermo Scientific Sorvall WX ultracentrifuges and the second fastest accel/decel rates in all other ultracentrifuges.

Rotor	Speed (rpm)	Time (h)
Fiberlite F50L-8x39	50,000	5.0
Fiberlite F65L-6x13.5	60,000	4.5

Table 1: Centrifugation conditions for RNA isolation

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