Total RNA Isolation Using Trizol and Qiagen RNAeasy Columns

Overview

This protocol is quick and works very well for preparing 20 to 40 ug of very clean, salt free, RNA. The RNA prepared from this protocol is ready for target preparation using the Ambion Message Amp II procedure to produce aminoallyl labeled cRNA.

Materials Required

- RNAase-free mortar and pestle: cover the mortar and pestle with aluminum foil and bake at least 3 hours at 180°C
- RNAase-free 1.5 or 2.0 mL (preferred) microfuge tubes
- Liquid nitrogen
- Microfuge
- RNAase-free pipette tips
- Qiagen RNAeasy Mini elute columns and buffers (Qiagen Cat # 74204)
- DEPC-treated H2O
- Trizol (Invitrogen)

Procedure

1. Homogenize tissue in liquid nitrogen. It is not necessary to homogenize large amounts of tissue as the Qiagen RNAeasy columns can only bind ~40 ug of RNA and overloading the column is not advised. If you are working with pooled samples you may find that you have more ground sample than you can use. It is convenient to have a small measuring device (teaspoon, grooved spatula, etc) to transfer the ground material directly to the Trizol in the microfuge tube.
    a. Chill mortar with ~100 mL of liquid nitrogen.
    b. Add frozen tissue after nitrogen is nearly completely evaporated.
    c. Grind tissue quickly but carefully.
    d. When liquid has fully evaporated, grind faster to produce a fine talc-like powder.
2. Add 1/8 to 1/4 teaspoon to 1.0 ml of Trizol. It is important to mix well immediately by vortexing and not allow tissue to thaw that is not in contact with the Trizol. You may want to prewarm the Trizol (35-40 C) so that it does not freeze when it comes into contact with the frozen tissue. This is the most critical step in the procedure as once the tissue is completely mixed with the Trizol, it is protected from RNAases.
3. Incubate for five minutes at room temperature (RT), votexing frequently.
4. Add 0.2 ml of chloroform to the Trizol, and vortex for 15 seconds.
5. Incubate for 1 minute at RT, vortex again for 15 seconds.
6. Centrifuge at 15,000 xg for 10 minutes to separate phases
7. Remove 200 ul from the top layer and add to 700 ul of Qiagen RLT buffer in a new tube. Remove the rest of the top layer and freeze at -20 to serve as a backup in case your initial yield is low.
8. To the 200 ul of sample now combined with 700 ul RLT buffer, add 500 ul of 96-100% ethanol. Mix well by vortexing but do not centrifuge.

9. Apply half of your sample (~700 ul) to a Qiagen MinElute spin column placed in a 2 ml microfuge tube. Spin 15 seconds at ~10,000 rpm. Discard flow through and repeat procedure with the second half of your sample.
10. Remove the Minelute column to a new 2 ml microfuge tube and add 500 ul of RPE to the spin column. Spin 15 seconds at ~10,000 RPM. Discard flow through.
11. Add 750 ul of 80% ethanol and spin at ~10,000 rpm for 15 seconds. Repeat this step a second time with another 750 ul of 80% ethanol. This step is repeated to ensure removal of all guanidine salts that may inhibit downstream applications.
12. Transfer the Minelute spin column to a new 2 ml microfuge tube. Spin for 5 minutes at top speed with the cap off. This ensures the removal of trace amounts of ethanol that may interfere with downstream applications.
13. To elute RNA, transfer spin column to a new, 1.5 ml microfuge tube. Elute with 10 ul of RNAase free water. Repeat with another 10 ul of RNAase free water. If you suspect low RNA concentration, you may elute with 12 ul of RNAase free water. It is desirable to have a concentration of ~ 1 ug/ul if possible. Check concentration on a gel or spectrophotometer. If your initial yield from the 200 ul is low, you may want to consider precipitating the remainder of your sample with an equal volume of isopropanol and resuspending in 200 ul of H2O for concentration using a minelute RNAeasy column.