Total RNA Extraction and Purification from Human Tissue Samples using TRIzol®

Materials:

TRIzol® Reagent (Invitrogen)

Ethanol (Fisher)

Isopropanol (Fisher)

Chloroform (Fisher)

Qiagen RNEasy MinElute Kit (Qiagen)

Homogenizer (Kinematica Hand Held Polytron Recommended)

Spectrophotometer (Nanodrop recommended)

RNAse-free 1.7mL tubes (Ambion)

Agilent Bioanalyzer and Eukaryote Total RNA nano chip setup

Glycogen (Invitrogen)

Mortar and Pestle (Fisher)

Scoopula Spatula (Fisher)

Tissue Forceps (Fisher)

RNAse Away (Fisher)

50ml Centrifuge Tubes (Fisher)

Razor Blades

Biohazard Considerations:

---This protocol describes the extraction of RNA from human tissues. It is important to consider the potential for infection or disease transmission by materials used in this procedure that contact the sample and/or the homogenate. Wear a lab coat, gloves, and proper eye protection if necessary.

--- To prevent transmission of human/animal tissues into the air or onto clothing or skin, carry out all homogenization and work with hazardous chemicals inside a biosafety hood. Homogenization can spray particles into the air and onto you.

---If using hazardous chemicals, the handling, collection, storage and disposal of all hazardous chemicals must be in accordance with Institute, EPA, OSHA and MWA rules and regulations.

--After working with bio-hazardous materials, thoroughly decontaminate the workspace and instruments according to Institutional protocol.

---Always use sterile equipment and practice sterile technique. Sterilize homogenizer generators, forceps and other equipment in ethanol and RNase Away frequently.

---Before starting wipe down the work surface and pipettes with RNAse Away to promote an RNAse-free environment.

Before Starting:

---Sterilize the homogenizer and generator by running it in RNAse free water, 75% ethanol and RNAse Away before use.

---Clean forceps and spatulas with 75% Ethanol, followed by RNAse Away, then dry using Kimwipes.

---Clean the mortar and pestle with RNAse free water, 75% ethanol and RNAse Away. Dry with Kimwipes.

---Place cleaned forceps and spatulas in the hood

---Dispense 3ml of TRIzol into a 50ml centrifuge tube and place on ice.

---Label two 2ml eppendorf tubes and place on ice.

RNA Extraction Procedure:

1.) Be sure the mortar and pestle, forceps, spatulas and other needed equipment are sterile and chilled on ice if necessary.

    Note: All tissue samples are immediately placed and stored in RNA*later*. RNA*later* protects the tissue from RNAse activity until it can be homogenized in TRIzol, so it is not necessary to keep everything frozen in liquid nitrogen.

2.) Remove tissue sample from freezer and allow it to thaw enough such that the tissue can be removed from the RNA*later*. Place the tissue into the mortar.

3.) Using a sterile razor blade, cut the tissue into small pieces.

4.) Using a spatula, scrape all the cut tissue pieces together in the mortar and place into the 50ml tube containing the TRIzol.

    Note: For larger tissue samples, it may be necessary to use more than 3mL of TRIzol. Add more TRIzol as necessary.

5.) Using an electric homogenizer with a heavy gauge generator (12mm), shred the tissue fully and completely. If homogenization takes a while, keep the tube on ice to prevent overheating and RNA degradation.

6.) Once all pieces of tissue have been ground, transfer ½ of the homogenate into each of the two 2ml eppendorf tubes.

    Note: If more than 3mL of TRIzol have been used, split the homogenate into as many 2ml eppendorf tubes as necessary.

7.) Using an electric homogenizer, homogenize the samples in TRIzol fully using a small gauge generator (5-7mm). Homogenize each sample tube at least 3 times for at least 1 minute each time. Keep the samples on ice in between each round of homogenization.

    Note: It is important to keep the samples cold. Excessive homogenization in small samples (1ml) can overheat the samples and cause RNA degradation.

8.) Let the samples stand for 5 minutes at room temperature. Cellular debris and insoluble material may start to pellet at the bottom of the tubes. **If you can see cellular debris and insoluble material start to accumulate at the bottom of the tube, or if the TRIzol becomes a muddy brown color instead of bright pink, continue on to step 9. If none of the above occurs (no debris starts to accumulate and the TRIzol remains pink), skip steps 9 and 10 and continue straight to step 11.**

9.) Centrifuge the homogenate at 10,000g for 15 minutes at 4ºC to spin out cellular debris and insoluble material.

10.) Carefully transfer the supernatant to fresh, RNase free 1.7ml tubes. If there is an

oily/fat layer on top, remove this layer carefully before transferring the supernatant.

11.) To each TRIzol supernatant, add 1ul of Glycogen (20ug/ul stock).

12.) Add 250ul Chloroform and shake vigorously by hand for 30 seconds. **Shake vigorously! If not shaken vigorously for a good 30 seconds, it will reduce RNA yields.**

13.) Let stand at room temperature for 10 minutes. You should start to see an initial phase separation.

14.) Centrifuge at 10,000g for 20 minutes at 4ºC.

15.) Transfer the aqueous layer (top, clear layer) to fresh RNase free 1.7ml tubes. Transfer as much as possible without interfering with the middle layer.

16.) Add an equal volume of isopropanol to each tube.

17.) Mix well and incubate **overnight** at -20ºC.

18.) Centrifuge at 10,000g for 20 minutes at 4ºC.

19.) Remove the isopropanol carefully from the pellet and add 1ml of cold 75% ethanol.

20.) Centrifuge at 7500g for 4 minutes at 4ºC.

21.) Carefully remove the ethanol, being careful not to disturb the pellet.

22.) Add another 1ml of cold 75% ethanol and centrifuge at 7500g for 4 minutes at 4ºC.

23.) Carefully remove the ethanol, and let the pellet air dry at room temperature for 5 minutes.

24.) Dissolve the RNA pellet in each tube in 100ul of RNase free water.

Note: Keep the RNA in each tube separate through the purification procedure below. The purified RNA will be combined at the end.

RNA Purification Procedure:

Before Starting:

---Add β-Mercaptoethanol to Buffer RLT

---Add 100% ethanol to Buffer RPE

---Make 80% ethanol in a 50ml centrifuge tube

---Follow all Institute, EPA, OSHA and MWA regulations for collection, handling and storage of hazardous waste.

1.) Add 350ul of Buffer RLT containing β-Mercaptoethanol to the 100ul of RNA in water and mix thoroughly by pipet.

2.) Add 250ul of 100% ethanol and mix thoroughly by pipet.

3.) Transfer all 700ul into an RNeasy MinElute spin column and centrifuge at 10,000g for 30 seconds.

4.) Discard the flow through and the collection tube.

5.) Place the spin column in a fresh 2ml collection tube and add 500ul of Buffer RPE

6.) Centrifuge at 10,000g for 30 seconds. Discard the flow through.

7.) Add 500ul of 80% ethanol and centrifuge at 10,000g for 2 minutes.

8.) Discard the flow through and place the spin column in a fresh 2ml collection tube.

9.) Centrifuge at 10,000g for 2 minutes to dry the filter membrane.

10.) Transfer the spin column to a fresh 1.7ml eppendorf tube and add 20ul of RNase Free water directly onto the filter membrane.

11.) Centrifuge at maximum speed for 1 minute.

12.) Add another 20ul of RNase free water to the filter membrane

13.) Centrifuge at maximum speed for 1 minute.

14.) Discard the spin column and place the RNA in 40ul of RNase free water onto ice.

15.) Combine the 40ul in each tube into one 1.7ml eppendorf tube containing 80ul.

Note: If the sample homogenate was split into more than two eppendorf tubes, combine them all in this step.

16.) After RNA analysis (Nanodrop and Bioanalyzer), attach a label to the RNA tube with important information and store at -80C.

Note: The resulting RNA contains large RNA's only. miRNA will be lost when using this protocol. If miRNA is desired, see the protocol "Total RNA and miRNA Extraction and Purification from Human Tissue Samples Using TRIzol®."

RNA Quantitation:

1.) Analyze the RNA on a spectrophotometer (Nanodrop recommended).

---A260/280 ratios should be between 1.8 and 2.1
---A260/230 ratios should be at least 1.9, over 2.0 is preferable.
---A260/280 ratios outside this range indicate DNA or protein contamination
---Low A260/280 ratios indicate carbohydrate, phenol, salt contamination

2.) Put the RNA on the Agilent Bioanalyzer to further confirm RNA purity and quality.