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

Materials:

Ethanol (Fisher)

Chloroform (Fisher)

Qiagen miRNEasy Mini Kit (Qiagen)

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

Mortar and Pestle (Fisher)

Scoopula Spatula (Fisher)

Tissue Forceps (Fisher)

RNAse Away (Fisher)

50ml Centrifuge Tubes (Fisher)

Razor Blades

Glycogen (Invitrogen)

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

---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.

    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 phenol, 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 a 50ml tube containing 3ml of 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.

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 tube, 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 could 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. Do not combine the tubes yet, keep the aqueous layers in separate tubes.

16.) Add 1 volume of 70% Ethanol to each tube.

17.) Mix well by vortexing.

Note: A precipitate may form, this is ok.

18.) Pipet 700ul of sample in each tube onto an RNeasy Mini column, including any precipitate that may have formed.

Note: The Qiagen RNeasy Mini columns have a limit as to how much material can be put through them. Be sure that your tissue sample does not exceed this limit. If it does, use multiple columns to process your sample. If you overload the columns, you will lose RNA! If in doubt or unsure, use multiple columns.

19.) Centrifuge at 10,000 rpm for 15-30 seconds.

Note: Repeat steps #18 and #19 if necessary until all sample has been processed.

20.) Remove the RNeasy Mini column from the collection tube and put it aside for later use. This column contains total RNA - do not throw it away!

21.) To the flow-through obtained from the centrifugation in step #19, add 450ul of 100% ethanol. Mix thoroughly by vortexing.

22.) Pipet 700ul of sample onto an RNeasy MinElute column.

23.) Centrifuge at 10,000 rpm for 15-30 seconds.

24.) Repeat steps #22 and #23 if necessary.

25.) Add 700ul Buffer RWT.

26.) Centrifuge at 10,000 rpm for 15-30 seconds.

27.) Add 500ul Buffer RPE.

28.) Centrifuge at 10,000 rpm for 15-30 seconds.

29.) Add 500ul of 80% ethanol.

30.) Centrifuge at 10,000 rpm for 2 minutes.

31.) Place the RNeasy MinElute column into a fresh collection tube and centrifuge at 10,000 rpm for 5 minutes to dry the column.

32.) Place the RNeasy MinElute column into a fresh 1.5mL eppendorf tube and add 14ul of RNase free water.

33.) Centrifuge at 10,000 rpm for 1 minute. Combine the sample from multiple columns (if multiple columns were used) together and place sample on ice.

34.) Obtain the RNeasy Mini column from step #20, place in a 2ml collection tube and add 700ul of Buffer RWT.

35.) Centrifuge at 10,000 rpm for 15-30 seconds.

36.) Add 500ul of Buffer RPE.

37.) Centrifuge at 10,000 rpm for 15-30 seconds.

38.) Repeat steps #36 and #37.

39.) Place the RNeasy Mini column in a fresh collection tube and centrifuge at 10,000 rpm for 2 minutes.

40.) Place the RNeasy Mini column into a fresh eppendorf tube and add 30ul of RNase free water.

41.) Centrifuge at 10,000 rpm for 1 minute.

42.) Combine the sample from multiple columns together (if multiple columns were used) and place the sample on ice.

Note: Keep the miRNA sample and the total RNA sample separate.

43.) Take an OD reading of the miRNA sample and the total RNA sample using a Nanodrop.

44.) Run the RNA samples on the Bioanalyzer using a Nano 6000 RNA chip and a Small RNA chip to assess quality.