## TRIZOL TISSUE RNA EXTRACTION PROTOCOL

(Modified from the manufacturer's instructions – refer to the Invitrogen TRIzol reagent protocol available online.)

Materials needed:

- 1. 50 mL conical tubes (5) filled with Milli-Q  $H_20$
- 2. 50 mL conical tube (1) filled with 70% ETOH made with Milli-Q  $H_2O$
- 3. RNaseZap (Ambion)
- 4. 75% ETOH made with UltraPure (nuclease-free) H<sub>2</sub>0
- 5. Isopropanol
- 6. Chloroform
- 7. TRIzol Reagent (Invitrogen)
- 8. Ice
- 9. Dry Ice
- 10. Tissue homogenizer
- 11. 1.5 mL microcentrifuge tubes
- 12. 5 mL microcentrifuge tubes
- 13. Filter tips

Preparation:

- 1. Weigh tissue samples (50-100 mg recommended) and transfer to a 5 mL microcentrifuge tube. Samples should be stored at -80°C until used and kept on dry ice during extraction until TRIzol reagent is added.
- 2. Wipe pipettes, homogenizer, and hood in the 7900 room thoroughly (1) with an RNaseZap-soaked paper towel and then (2) with a water-dampened paper towel. Repeat (2) and let them air dry.
- 3. Set up the 50 ml conical tubes in a rack in the following order: Milli-Q  $H_20$ , 70% ETOH, Milli-Q  $H_20$ , Milli-Q  $H_20$ , Milli-Q  $H_20$
- 4. Place a homogenization probe into the homogenizer and wash the probe in 70% ETOH followed by 2 waters. When using the homogenizer, move up in speed **slowly** and try not to use speeds higher than 3. If the homogenizer gets hot, stop using and let it cool down. Make sure that the upper venting hole is not submerged in liquid while the homogenizer is on.

## Extraction:

- 1. Place tube with sample on ice and add appropriate amount of TRIzol reagent into the tube 1 ml/50-100 mg foreskin tissue and 3 ml/50-100 mg placental tissue recommended. The exact amount of TRIzol to be added should be determined empirically based on tissue weight. The sample volume should not exceed 10% of the volume of TRIzol reagent used for homogenization.
- 2. Homogenize tissue <u>on ice</u> by placing the lower portion of the homogenizer probe in TRIzol solution until no visible particles remain. Incomplete homogenization results in significantly reduced RNA yields. Keep homogenized samples on ice.
- 3. When homogenization is complete, decrease the speed of the probe, gently tap the probe against the side of the tube, and remove from the solution. Blot homogenizer with paper towel to remove excess Trizol solution (make sure there is no tissue left in the probe). Wash the probe in the first water for a few seconds, and then go through the 70% ETOH and 3 waters. Blot the probe with paper towel after each wash. Now you are ready to move on to a new sample.
- 4. Once all samples are homogenized, take them out of the ice and let incubate at room temperature for 5 minutes. During this 5 minute incubation, transfer the homogenate into a 1.5 mL labeled microcentrifuge tube (1 ml each). If you used more than 1 ml of Trizol for the initial homogenate to be transferred.
- 5. After the incubation, add 200 ul chloroform to each sample, shake vigorously by hand for 15 seconds and incubate at room temperature for 2-3 minutes.
- 6. Centrifuge 12,000 x g for 15 minutes at 4°C. Note: The mixture separates into a lower red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The upper aqueous phase is ~50% of the total volume.
- 7. Transfer the aqueous phase of the sample into a new labeled 1.5 mL microcentrifuge tube by angling the tube at 45° and pipetting the solution out. Avoid drawing any of the interphase or organic layer into the pipette when removing the aqueous phase.
- 8. Add 0.5 mL of 100% isopropanol to the aqueous phase.

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- 9. Incubate at room temperature for 10 minutes.
- 10. Centrifuge at  $12,000 \times g$  for 10 minutes at 4°C. Note: The RNA is often invisible prior to centrifugation, and forms a gel-like pellet on the side and bottom of the tube.
- 11. Remove the supernatant from the tube, leaving only the RNA pellet.
- 12. Add 1 mL 75% ethanol to the pellet and Centrifuge the tube at 7500 × g for 5 minutes at 4°C.
- 13. Pour off the supernatant into a waste container containing ethanol. If the pellet is visible, pipette off any excess ethanol and let air dry for 10-15 minutes. Pellet should be almost completely dry, but a little moisture will help it dissolve better.
- 14. Resuspend the RNA pellet in 10-20  $\mu$ l RNase-free water (the exact amount should be determined based on pellet size). Leave on ice for 10-15 minutes and then mix by passing the solution up and down through a pipette tip.
- 15. Store samples at -80°C.
- 16. Wash the homogenizer probe as stated in Step 3 and then perform additional wash with fresh water. Blot the probe with paper towel. Bleach the water used for washing for half an hour and then pour down the sink. Wipe the hood and the homogenizer with 70% ETOH.