RNA extraction with TRIzol

(protocol adapted from Invitrogen TRIzol Cat. no. 15596026)

Reagents and Equipment

- TRIzol (Invitrogen Cat. no. 15596026)
- *(Optional)* RNase-free glycogen (Invitrogen Cat. no. AM9510)
- 100% ethanol (AQA Ethyl Alcohol (absolute) ACS Grade 5L no. AL-1551-5000)
- Chloroform (Sigma Cat. no. C2432)
- Isopropanol (Sigma Cat. no. I9516)
- UltraPure Distilled Water (Invitrogen Cat. No. 10977-015)
- Pestle (Kimble Chase motor cordless no. 749540)
- Centrifuge and rotor capable of reaching 12,000 × g and 4°C

Protocol for Tissues (e.g., macroareas, whole brain)

Perform RNA isolation immediately after sample collection or quick-freeze samples immediately after collection and store at –80ºC or in liquid nitrogen, or in RNA*later* at –20ºC until RNA isolation.

1. Disrupt and homogenize 50-100mg of tissue in **1 mL** TRIzol Lysis Reagent (**1,5** ml eppendorf; proportion tissue:TRI of 1:10) using pestle (Samples always on ice - 1 cycle of 45 s, repeat if any tissue is still visible. For samples stored in RNA*later* do 1 cycle of **60** s).

2. Incubate sample at RT for 5 min to allow complete dissociation of the nucleoproteins complex.

3. Add 200 µl chloroform (kept at 4ºC; proportion Chlor:TRI of 1:5), and shake vigorously for 15 s (use fume hood)

4. Incubate sample at RT for 3 min.

5. Centrifuge the sample for 15 min at 12.000 × g (max. rpm) at 4ºC.

Warning: The mixture separates into a lower red phenol-chloroform, interphase, and a colorless upper aqueous phase. Don't jostle the tubes after spinning and don't take every bit of supernatant of the interphase to avoid gDNA contamination. After step 5 samples should be handled on ice.

6. Transfer upper, aqueous phase to a new tube (**1,5** ml eppendorf) by angling the tube at 45ºC.

7. *(Optional)* If the starting sample is small (<10 mg of tissue), add 5–10 µg of RNase-free glycogen as a carrier to the aqueous phase.

8. Add 0.5ml of cold isopropanol to the aqueous phase (proportion Isop:TRI of 1:2), and incubate for 10 minutes at 4ºC

9. Centrifuge for 10 minutes at 12,000 × g at 4°C.

Note: Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.

10. Discard the supernatant, and resuspend the pellet in 1 mL of 75% ethanol (proportion ETOH:TRI of 1:1).

11. Vortex the sample briefly, then centrifuge for 5 minutes at 12,000 × g at 4°C.

12. Discard the supernatant and air-dry the RNA pellet for 5-10 min.

13. Resuspend the pellet in 20–50 µL of RNase-free water, gently mix, and measure concentration in the nanodrop. Store at –80 ºC.

Version 24.05.22, Sara Cardoso