LSOP Title	RNA extraction
LSOP No.	LSOP39
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste*
Risk Assessments	
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Date Approved	11/10/2021
Date Effective	30/06/2021
Next Review Date	30/06/2026
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1.0 Scope

This procedure covers the RNA extraction method that includes; homogenization, phase separation, RNA isolation, RNA wash, Digestion of RNA and clean-up of RNA.

This LSOP does not cover the SDS/CTAB method for RNA extraction.

2.0 Materials and Equipment

1. Liquid Nitrogen
2. Pestle
3. Harvest Tubes

4. TRIzol
5. Vortex

6. Chloroform
7. Centrifuge
8. Microcentrifuge tubes
9. Pipette and Pipette Tips

10. Isopropanol

11. Ethanol
12. Water
13. Buffer RDD
14. DNase
15. Qiagen RNeasy MinElute Cleanup kit

    a. RLT buffer
    b. RPE buffer
    c. Pink spin column

3.0 Prescribed Actions

Homogenization

1. Retrieve tissue samples from -80 °C and place in liquid nitrogen.
2. Cool down the pestle in liquid nitrogen just before use
3. Using liquid nitrogen and a pestle, grind samples in 2 mL screw-top harvest tubes.

Phase separation: IN FUME HOOD

4. With pestle still in 2 mL tube with sample, at room temperature add 500 µL TRIzol reagent to sample, and grind sample further.
5. Vortex for 30 seconds.
6. Incubate sample for 5 minutes at room temperature to allow complete dissociation of the nucleoprotein complexes.

7. Add 100 µL chloroform Vortex sample for 15 seconds (the suspension will become whitish).
8. Incubate sample for 5 minutes at room temperature.
9. At 4°C, centrifuge the sample at 12 000 g (rcf) for 15 minutes (use centrifuge in cold room).

    NB: After centrifugation, the mixture separates into a lower red phenol/chloroform phase, an interphase, and a colourless upper aqueous

phase. The RNA is exclusively in the upper aqueous phase, which is ~ 50% of the total volume.

10. Transfer the aqueous phase of the sample into a new 1.5 mL microcentrifuge tube by angling the tube at 45° and pipetting the solution out (approximately 300 µL). Avoid the interphase and organic lower phase.
11. The remaining interphase and lower phase should be discarded in appropriate waste container.

RNA Isolation

12. Add 250 µL isopropanol to the aqueous phase
13. Incubate at room temperature for 5 minutes.
14. Centrifuge at 12 000 g (rcf) for 10 minutes
15. The RNA will form a pellet on the side and bottom of the microcentrifuge tube.

RNA Wash

16. Remove the supernatant from the tube, leaving only the RNA pellet.
17. The supernatant waste should be discarded in appropriate waste container.

18. Add 1 mL (fresh) 70 % ethanol to the pellet
19. Centrifuge at 8000 g (rcf) for 5 minutes
20. Remove the supernatant from the tube and air dry the RNA pellet for 10 minutes. DO NOT use vacuum centrifuge.
21. Resuspend the RNA pellet in 100 µL sterile water *
22. Store samples at -80°C if not proceeding to the next step

NB: If you want to do a DNase digestion of your RNA before using the cleanup kit (the cleanup kit removes most DNA, so only do DNase digestion if using downstream applications sensitive to small amounts of DNA), do not resuspend in 100 µL sterile water, instead follow the instructions below.

Genomic DNase Digestion of RNA

1. Resuspend pellet in 87.5 µL sterile water
2. Add 10 µL Buffer RDD and 2.5 µL DNase 1 stock solution

   NB: Buffer RDD is stored in the fridge

   NB: DNase 1 stock solution is stored in aliquots in the freezer at -20°C for up to 9 months, once defrosted each aliquot should be stored in the fridge at 2-8°C for up to 6 weeks
3. Incubate at room temperature for 10 minutes
4. Proceed straight on to the Qiagen RNeasy MinElute Cleanup kit.

RNA clean up using Qiagen RNeasy MinElute cleanup kit

5. To the 100 µl of RNA:

6. Add 350 µl RLT buffer (containing 4.5 µl b-mercaptoethanol) and vortex

7. Add 250 µl 96-100% Ethanol and mix by pipetting
8. Add solution (~700 µL) to a Qiagen pink spin column (stored in fridge).
9. Centrifuge at 8000 rcf for 15 seconds
10. Discard the flow-through (in appropriate waste container) and collection tube
11. Place spin column in a new 2 mL collection tube
12. Add 500 µL Buffer RPE

13. Check ethanol has been added
14. Close lid gently and centrifuge at 8000 rcf for 15 seconds
15. Discard the flow through (in appropriate waste container)

16. Add 500 µL 80% ethanol
17. Close lid gently and centrifuge at 8000 rcf for 2 minutes
18. Discard flow through and collection tube

19. Place spin column in a new 2 mL collection tube
20. Open lid and centrifuge at full speed (16,100 rcf) for 5 minutes
21. Discard flow through and collection tube
22. Place spin column in 1.5 mL collection rube
23. Add 14 µL RNase-free water directly to the centre of the spin column
24. Close lid gently and centrifuge at full speed (16,100 rcf) for 1 minute
25. Store samples at -80°C.