Total RNA extraction protocol for individual ants using the Bioline Isolate II kit

Modified from the manufacturer’s protocol available at https://www.bioline.com/uk/downloads/dl/file/id/3799/isolate\_ii\_rna\_dna\_protein\_kit\_manual.pdf

Required

-RNase-free 1.5 ml Eppendorf tubes

-Liquid nitrogen

Lysate preparation

1. Transfer starting material to an RNase-free 1.5 ml Eppendorf tube *without thawing*.
2. Immediately submerge the (closed) tube in liquid nitrogen,
3. Using long forceps, remove the tube from the liquid nitrogen. Quickly open the lid and insert a clean, RNase-free pestle. (If the material is not at the bottom of the tube, push it to the bottom using the pestle). Immediately immerse the bottom half of the tube in liquid nitrogen, using the forceps to grip the tube around the arm of the lid. (Avoid allowing liquid nitrogen into the open tube as the starting material may float away). The liquid nitrogen will stop bubbling when the tube has been cooled. Remove the tube and grind *hard* – the starting material should crumble easily into a powder. Immediately submerge the bottom of the tube in liquid nitrogen as before, and then remove and continue grinding. It is important that the starting material is completely ground up and that it *does not thaw*.
4. Add **300 ul of Lysis Buffer TX** and continue to grind until the sample has been homogenised.
5. Vortex for 5 seconds.
6. *Optional* – **incubate at 55 C for 10 minutes**.
7. Centrifuge at 14,000 x g for 2 minutes to pellet any cell debris.
8. Immediately move to next section (below).

RNA isolation

1. Assemble an Isolate II DNA column(white ring) with a collection tube.
2. Transfer supernatant onto the column and centrifuge at **14,000 x g for 1 minute**.
3. Inspect the column and tube, looking for any un-transferred lysate. If any lysate remains in the column and has not passed through into the collection tube, spin again at **14,000 x g for 1 minute**.
4. Place the collection tube on ice or at -20 C. Discard the DNA column.
5. Add **180 ul of 96% - 100% ethanol** to the collection tube. Mix by vortexing.
6. Assemble an Isolate II RNA/protein column (black ring) with a new collection tube.
7. Transfer the contents of the old collection tube to the new column and spin at **>=3,500 x g for 1 minute**.
8. Inspect the column and tube, looking for any un-transferred liquid. If any liquid remains in the column and has not passed through into the collection tube, spin again at **14,000 x g for 1 minute**.
9. Discard the contents of the collection tube and re-assemble with the column.
10. *Optional* – refer to Appendix B of the Isolate II manual for an extra DNase I treatment.
11. Apply **400 ul of Wash Buffer W1** to the column and centrifuge at **14,000 x g for 1 minute**.
12. Inspect the column and tube, looking for any un-transferred liquid. If any liquid remains in the column and has not passed through into the collection tube, spin again at **14,000 x g for 1 minute**.
13. Discard flow-through and re-assemble the column and tube.
14. Wash the column a second time with **400 ul Wash Buffer W1** and spin at **14,000 x g for 1 minute**. As before, spin again if not all liquid transfers to the collection tube.
15. Discard the flow-through and reassemble the spin column and tube.
16. Third wash with another **400 ul Wash Buffer W1** and centrifuge at **14,000 x g for 1 minute** (again spinning a second time if necessary).
17. Discard flow-through and reassemble column and tube.
18. Spin the column for **2 minutes at 14,000 x g** in order to thoroughly dry the column. Check to see if any liquid is left on the column, spin again if necessary.
19. Discard the collection tube.
20. Place the column into a fresh 1.7 ml Elution Tube (supplied in kit).
21. Add **50 ul of RNA Elution Buffer** to the column.
22. Centrifuge at **200 x g for 2 minutes**, followed by **14,000 x g for 1 minute**. If any liquid remains visible on the column, spin again at **14,000 x g for 1 minute.**
23. The eluted RNA can be stored at -20 C for up to 3 days, or at -80 for long-term storage. In either case it is vital to *immediately transfer the tube to the chosen storage place*.