QIAGEN RNA extraction from parts of *Drosophila* samples stored in RNAlater

RNAlater is meant for animal organs, such as kidney. The crystals are supposed to be removed before using the tissue. For small samples, such as *Drosophila* testes or accessory glands, the salts are removed by adding an equal volume of PBS to dissolve the salts. Most are then removed after spinning the samples, by pipetting off the supernatant.

Dissections of *Drosophila* are best done on an ice block covered with a black rubbish bag, in RNAlater. The dissected tissues are then moved to 200 ul RNAlater per sample.

This protocol produces close to 1 mg clean total RNA from 100 *Drosophila* female reproductive tracts (excluding ovaries).

Before Starting

- Make sure RPE has had ethanol added.
- Make QIAGEN DNAse
    - Add 550 ul RNase free H2O to the glass vial using a syringe.
    - Invert the vial to mix the contents
    - Remove the foil cover, open then lid and pipette off the solution to aliquots to store at –20oC, e.g. 40 ul for 4 samples at a time.
- Add 10 ul beta-mercaptoethanol (dangerous) per 1 ml RLT in a fume hood.
    - 14 ul in 1400 ul RLT for 4 samples
- Prepare fresh 70% ethanol
    - 420 ul RNase free H2O and 980 ul Ethanol.
    - Label new tubes and add 350 ul of freshly made 70% EtOH in each tube.

Procedure

Assumes 4 samples are done at a time, and they started in 200 ul RNAlater.

1. Add equal to RNAlater volume (200 ul) PBS to RNAlater samples, invert to mix and dissolve crystals, spin for 10 min to pellet the tissues.
2. Remove as much of the supernatant as possible.
3. Add 200 ul RLT.
4. Homogenise with spinning machine. White tips work best.
5. Add 150 ul RLT to each vial.
6. Centrifuge for 3 min.
7. Transfer supernatant to already labelled tube with 600 ul 70% EtOH, mix by pipetting,
8. Transfer 700 ul at a time, along with precipitate, to RNeasy spin column inside a 2ml tube. Close lid and spin for 15s at > 10,000 rpm.
9. Discard flow-through. Repeat until all sample is used.
10. (Optional) on-column DNA digestion happens here. If so
    1. Add 350 ul RW1, close lid and roll/invert tube, spin for 15 sec, discard flow though.
    2. DNase digestion
        1. Make DNase from pre made frozen eppendorf with 40 ul DNase mixed with 320 ul buffer RDD.
        2. Pipette 80 ul DNase mix to the each spin column membrane
        3. Incubate for 15 min at room temperature.

3. Add 350 ul RW1, close lid and roll/invert tube, spin for 15 sec, discard flow though.

11. (only if did not do DNase) Add 700 ul RW1. Spin for 15 sec at 10,000 rpm. Discard flow-through.
12. Add 500 ul RPE, close lid and roll/invert tube, spin for 15 sec, discard flow though.
13. Add 500 ul RPE, close lid and roll/invert tube. Spin for **2 min** at 10,000 rpm.
14. Place RNeasy column in new 2ml collection tube and spin for 5 min with open lids to completely dry membrane.
15. Place spin column in 1.5 ml collection tube. Add 30–50 ul RNase-free water on column membrane. Spin for 1 min to elute RNA.
16. (Optional) Repeat to increase yield.