RNAzol RT/Direct-zol RNA extraction and purification protocol (total RNA)

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Protocols:

<https://www.mrcgene.com/wp-content/uploads/2017/04/RNAzolRTMarch2017.pdf>

<https://files.zymoresearch.com/protocols/_r2060_r2061_r2062_r2063_direct-zol_rna_microprep.pdf>

(Before start) Prepare DNase:

5 ul DNase I (6 U/ul), 35 ul DNA digestion buffer – amount for each sample

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1. Cut 15 mL falcon tubes in half at the 7 mL mark, put bottom halves (without cap) on ice
2. Aliquot 2.5 mL of RNAzol RT into the tubes and store on ice.
3. Blot oyster tissue as dry as possible on brown paper towel
4. Transfer 300-500 mg of tissue to tubes containing RNAzol RT.
5. Homogenize immediately with disposable pestle.
6. Transfer to intact 15 mL falcon tubes, vortex 15s.
7. Add 1.2 mL of 0.1% DEPC-treated H2O to tubes.
8. Vortex 15s.
9. Incubate at room temperature (RT) for 15mins.
10. Centrifuge 9,500 RPM on the large centrifuge with the SL-50 rotor (in the glass front fridge in 213) for 15mins @ RT.
11. Transfer 750uL of supernatant (do not disturb pellet) to sterile 1.7mL snap-cap tube. (Keep remaining sample on ice and eventually move to the box in the -80 for our bigger samples.)
12. Add 750uL of 2-propanol (isopropanol) or 100% ethanol.
13. Vortex 5s.
14. Incubate @ RT for 5mins (*might not be needed*).
15. Transfer mixture into Zymo-Spin IC column w/ collection tube
16. Centrifuge 10,000g for 30s (or until all cleared)
17. Transfer column into a new collection tube, discard flow-through
18. Add 400uL RNA Wash Buffer to column
19. Centrifuge 10,000g for 30s
20. Add 40uL of DNase solution directly to column
21. Incubate @ RT for 15mins
22. Add 400uL Direct-zol RNA PreWash to column
23. Centrifuge 10,000g for 30s
24. Discard flow-through. Repeat steps 21-22.
25. Discard flow-through. Add 700ul RNA Wash Buffer.
26. Centrifuge 10,000g for 1 min
27. Transfer column to RNase-free tube
28. Add 50uL of DNase/RNase-Free water
29. Centrifuge 10,000g for 30 sec.
30. Keep sample on ice for short-term storage (i.e., no more than 2hrs); Store @ -80C. Do Qubit and nanodrop analyses if there’s time.