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. Aliquot 500uL of RNAzol RT to pestle tubes and store on ice.
2. Transfer tissue to pestle tubes containing RNAzol RT.
3. Homogenize immediately with disposable pestle.
4. Immediately add an additional 500uL of RNAzol RT to the pestle tube.
5. Vortex 15s.
6. Add 400uL of 0.1% DEPC-treated H2O.
7. Vortex 15s.
8. Incubate at room temperature (RT) for 15mins.
9. Centrifuge 12,000g for 15mins @ RT.
10. Transfer 750uL of supernatant (do not disturb pellet) to sterile 1.7mL snap-cap tube. (Discard remaining liquid in RNAzol RT Hazardous Waste container in fume hood. Leave the old tube open in the fume hood overnight and then discard it in regular trash.)
11. Add 750uL of 2-propanol (isopropanol) or 100% ethanol.
12. Vortex 5s.
13. Incubate @ RT for 5mins (*might not be needed*).
14. Transfer mixture into Zymo-Spin IC column w/ collection tube
15. Centrifuge 10,000g for 30s (or until all cleared)
16. Transfer column into a new collection tube, discard flow-through
17. Add 400uL RNA Wash Buffer to column
18. Centrifuge 10,000g for 30s
19. Add 40uL of DNase solution directly to column
20. Incubate @ RT for 15mins
21. Add 400uL Direct-zol RNA PreWash to column
22. Centrifuge 10,000g for 30s
23. Discard flow-through. Repeat steps 21-22.
24. Discard flow-through. Add 700ul RNA Wash Buffer.
25. Centrifuge 10,000g for 1 min
26. Transfer column to RNase-free tube
27. Add 50uL of DNase/RNase-Free water
28. Centrifuge 10,000g for 30 sec.
29. Keep sample on ice for short-term storage (i.e., no more than 2hrs); Store @ -80C.