

# RNAzol RT extractions protocol

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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 H<sub>2</sub>O.
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).
12. Vortex 5s.
13. Incubate @ RT for 15mins.
14. Centrifuge 12,000g for 10mins @ RT.
15. Discard supernatant; do not disturb pellet.
16. Add 400uL of 75% ethanol (made w/ DEPC-treated H<sub>2</sub>O)
17. Centrifuge 4,000g for 3mins @ RT.
18. Removal residual ethanol. Add 400 uL of 75% ethanol.
19. Centrifuge 4,000g for 3mins @ RT.
20. Remove residual ethanol. Add 50uL 0.1% DEPC-H<sub>2</sub>O.
21. Centrifuge 4,000g for 1min @ RT.
22. Removal residual DEPC-H<sub>2</sub>O & ethanol.
23. Immediately resuspend pellet in appropriate volume of 0.1% DEPC-H<sub>2</sub>O (volume is dependent upon pellet size, but 50uL is usually sufficient).
24. Keep sample on ice for short-term storage (i.e., no more than 2hrs); Store @ -80C.