**Zymo Direct-zol RNA MiniPrep Extraction Protocol**

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* All centrifugation steps are performed at **room temperature** and **16,000 x *g***.
* Prior to first use, DNase I should be reconstituted per instructions on tube and stored as frozen aliquots to minimize freeze/thaw cycles.
* Prior to first use, add ethanol to buffer concentrates per instructions on bottles.

1. Prepare DNase prior to extraction. Add 5 µL DNase I and 75 µL DNase Digestion Buffer per sample (e.g. for 24 samples use 120 µL DNase I and 1800 µL Digestion Buffer) to an RNase-free tube and mix thoroughly by gentle inversion. **Keep mixture on ice.**
2. Heat DNase/RNase-Free Water tube on a heat block at 55°C.
3. Scrape tissue from coral fragment and place into a 2 mL SafeLock tube with 0.1 mL (~ 0.075 g) of 0.5 mm glass beads.
4. Add 600 µL TRIzol.
5. Bead beat for 3 min (6 m/s, three 60 s intervals w/ 2 min cool down between).
6. Centrifuge lysate for 2 min to pellet beads and debris. Transfer supernatant (400 µL) to a 1.5 mL tube.
   1. Supernatant should be transparent, not murky or viscous. If not, increase TRIzol until solution clears.
7. Add equal volume of ethanol (400 µL) and mix thoroughly. Transfer to Zymo-Spin Column in a collection tube and centrifuge for 30 s. Discard flow-through.
8. Add 400 µL RNA Wash Buffer to the column and centrifuge for 30 s. Discard flow-through.
9. Add 80 µL prepared DNase to each column.
10. Incubate at room temperature for 15 min.
11. Add 400 µL Direct-zol RNA PreWash to the column and centrifuge 30 s. Discard the flow-through and **repeat this step and discard flow-through**.
12. Add 700 μl RNA Wash Buffer to the column and centrifuge for 30 s and discard flow-through. Centrifuge for another 2 min to completely remove wash buffer. Transfer the column carefully into a new catch tube. Avoid contact of flow through and spin column.
13. To elute RNA, add 50 μL of heated DNase/RNase-Free Water directly to the column, incubate for 1–2 min, and centrifuge for 1 min.
14. Transfer eluted and purified RNA to a 0.6 mL tube and store at -80°C.