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

Written by R. Eckert

Updated: 08.25.20 M. Studivan

All centrifugation steps are performed at **room temperature** and **16,000 x *g*** unless specified.

Prior to first use, DNase I should be reconstituted in 275 µL of DNase/RNase-Free Water and stored as frozen aliquots to minimize freeze/thaw cycles. Add 5 µL DNase I per sample (e.g. for 24 samples use 125 µL DNase I) to 2 mL tubes. Freeze until needed.

Prior to first use, add ethanol to buffer concentrates per instructions on bottles.

1. Thaw DNase I aliquot on ice**.**
2. Heat DNase/RNase-Free Water tube on a heat block at 60°C.
3. Optional: Scrape tissue from coral fragment and place into a 2 mL screw-top tube with 0.2 mL (~ 0.075 g) of 0.5 mm glass beads and 1.0 mL TRIzol.
4. Bead beat for 2 min (6 m/s, 60 s intervals w/ 2 min cool down on ice in between). Remove from ice and incubate at room temperature for 5 min.
5. Centrifuge lysate for 2 min to pellet beads, debris, DNA, and polysaccharides. Transfer 800 µL of supernatant to new 2.0 mL tube.
6. Add equal volume of ethanol (800 µL) and mix thoroughly. Transfer 800 µL aliquot to Zymo-Spin Column in a collection tube and centrifuge for 30 s, empty flow-through, then repeat for remaining 800 µL. Transfer spin column to a **new** **collection tube**.
7. Add 400 µl RNA Wash Buffer to the column and centrifuge for 30 s.
8. Prepare DNase master mix in the DNase I aliquot tube by adding 75 µL DNA Digestion Buffer per sample (1.875 mL for 24 samples) and mix by pipetting.
9. Add 80 µL of DNase mix directly to each column.
10. Incubate at room temperature for 25 min.
11. Add 400 µl Direct-zol RNA PreWash to the column and centrifuge. Discard the flow-through and **repeat this step**.
12. Add 700 µL RNA Wash Buffer to the column and centrifuge for 30 sec. Empty flow-through, then centrifuge again for 2 min to ensure complete removal of the wash buffer. Transfer the column carefully into a new catch tube. Avoid contact of flow through and spin column tip.
13. To elute RNA, add 50 µL of heated DNase/RNase-Free Water directly to the column, incubate for 2 min, and centrifuge for 30 s.
14. Store on ice for purification.

**Zymo RNA Clean & Concentrator-5 Purification Protocol**

Written by M. Studivan

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All centrifugation steps are performed at **room temperature** and **16,000 x *g*** unless specified.

Prior to first use, add ethanol to buffer concentrates per instructions on bottles.

1. Optional: Adjust sample volume to 50 ul with DNase/RNase-Free Water.
2. Add 2 volumes (100 µL) of RNA Binding Buffer to each sample and mix by pipetting.
3. Add an equal volume (150 µL) of 100% ethanol and mix by pipetting.
4. Transfer to Zymo spin columns and centrifuge for 30 sec. Empty flow-through.
5. Add 400 µL of RNA Prep Buffer to the column and centrifuge for 30 sec. Empty flow-through.
6. Add 700 µL of RNA Wash Buffer to the column and centrifuge for 30 sec. Empty flow-through.
7. Add 400 µL of RNA Wash Buffer to the column and centrifuge for 30 sec. Empty flow-through, then centrifuge again for 2 min to ensure complete removal of the wash buffer. Transfer the column carefully into a new catch tube. Avoid contact of flow through and spin column tip.
8. Add 20 µL of DNase/RNase-Free Water heated to 60 ºC directly to the filter and incubate for 2 min. Centrifuge for 30 sec.
9. Transfer purified RNA to a 0.5 mL tube and store at -80 ºC.

**Direct-zol Tube Prep: RNA Clean & Concentrator Tube Prep:**

2.0 mL bead tubes with 1 mL TRIzol Zymo Spin Column w/ collection tube

2.0 mL tube for homogenate 1.5 mL catch tube

Zymo Spin Column w/ collection tube 0.5 mL Safe-Lock tube

New collection tube

1.5 mL catch tube