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

Written by R. Eckert

Updated: 11.16.23 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 Zymo bead tube with 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 700 µL of supernatant to new 2.0 mL tube.
6. Add equal volume of ethanol (700 µL) and mix thoroughly. Transfer 700 µL aliquot to Zymo-Spin Column in a collection tube and centrifuge for 30 s, discard flow-through, then repeat for remaining 700 µL. Transfer spin column to a **new** **collection tube**.
7. Add 400 µl RNA Wash Buffer to the column, invert tubes, and centrifuge for 30 s. Discard flow-through and dab collection tube on a KimWipe.
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-zolRNA PreWashto the column and centrifuge. Discard the flow-through and dab collection tube on a KimWipe. **Repeat this step**.
12. Add 700 µL RNA Wash Buffer to the column, invert columns only, and centrifuge 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 5 min, and centrifuge at 19,000 x *g* for 1 min.
14. Store eluted RNA **on ice** until the next kit protocol.

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

Written by M. Studivan

Updated: 11.16.23 M. Studivan

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 vortex.
3. Add an equal volume (150 µL) of 100% ethanol and mix by vortex.
4. Transfer to Zymo-Spin IC Column in a collection tube and centrifuge for 30 sec. Discard flow-through and dab collection tube on a KimWipe.
5. Add 400 µL of RNA Prep Buffer to the column and centrifuge for 30 sec. Discard flow-through and dab collection tube on a KimWipe.
6. Add 700 µL of RNA Wash Buffer to the column, invert columns only, and centrifuge for 30 sec. Discard flow-through and dab collection tube on a KimWipe.
7. Add 400 µL of RNA Wash Buffer to the column and centrifuge for 2 min to ensure complete removal of the wash buffer. Transfer the column carefully into a new 1.5 mL catch tube. Avoid contact of flow through and spin column tip.
8. Add 25 µL of DNase/RNase-Free Water heated to 60 ºC directly to the filter and incubate for 5 min. Centrifuge at 19,000 x *g* for 1 min.
9. Nanodrop eluted RNA, blanking using the same DNase/RNase-Free Water used for elution.
10. Store eluted RNA at -80 ºC.

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

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

2.0 mL tube for homogenate 1.5 mL catch tube

Zymo Spin Column w/ collection tube

New collection tube

1.5 mL catch tube