**Zymo DNA/RNA ZymoBIOMICS Extraction Protocol**

Written by M. Studivan

Updated: 10.19.23 M. Studivan

All centrifugation steps are performed at **room temperature** and **16,000 x *g* (RCF)**for 30 seconds 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 Zymo DNA/RNA Shield.
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 1 min to pellet beads, debris, and polysaccharides. Transfer 350 µL of supernatant to new 2.0 mL tube.
6. Add equal volume of DNA/RNA Lysis Buffer (350 µL) and mix thoroughly. Transfer 700 µL aliquot to Spin-Away Filter (**yellow**) in a collection tube and centrifuge for 30 s. Transfer spin column to a **new** **collection tube for DNA** (skip to step 12), and **retain the flow-through for RNA** (steps 7-11, then continue).

**RNA:**

1. Add equal volume of ethanol (700 µL) to the RNA flow-through and mix by pipetting. Transfer 700 µL aliquot to Zymo-Spin IIICG Column (**green**) in a collection tube and centrifuge for 30 s, empty flow-through and dab on KimWipe, then repeat for remaining 700 µL.
2. Add 400 µl DNA/RNA Wash Buffer to the column, invert columns only, centrifuge for 30 s, and empty flow-through and dab.
3. Prepare DNase master mix in a DNase I aliquot tube by adding 75 µL DNA Digestion Buffer and 5 uL reconstituted DNase I per sample (1.875 mL for 24 samples) and mix by pipetting.
4. Add 80 µL of DNase mix directly to each column.
5. Incubate at room temperature for 25 min.
6. Add 400 µl DNA/RNA Prep Bufferto the column and centrifuge. Discard the flow-through and dab.
7. Add 700 µL DNA/RNA Wash Buffer to the column, invert columns only, and centrifuge. Discard the flow-through.
8. Add 400 µL DNA/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 catch tube. Avoid contact of flow through and spin column tip.
9. To elute, 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.
10. Store eluted RNA on ice for purification or inhibitor removal, or at -80 ºC.
11. Optional: Add 600 µL of HRC Prep Solution to Zymo-Spin III-HRC Filter in a collection tube, centrifuge at 8,000 x *g* for 3 min, discard collection tube, and place filter in a new catch tube.
12. Optional: Transfer eluted DNA/RNA into prepared Zymo-Spin III-HRC Filter and centrifuge at 16,000 x *g* for 3 min.

**ZymoBIOMICS Tube Prep:** (optional) **HRC Tube Prep:**

2.0 mL bead tube with 1 mL DNA/RNA Shield Zymo-Spin III-HRC Filter w/ collection tube

2.0 mL tube for homogenate 1.5 mL catch tube for RNA

Spin-Away Filter (yellow) w/ collection tube 1.5 mL catch tube for DNA

New collection tube for yellow filter

Zymo-Spin IIICG Column (green) w/ collection tube

1.5 mL catch tube for RNA

1.5 mL catch tube for DNA

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

Written by M. Studivan

Updated: 10.19.23 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. 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, invert columns, and centrifuge for 30 sec. Empty flow-through.
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 for 1 min.
9. Store eluted RNA **on ice** until long-term storage.
10. Nanodrop eluted RNA/DNA, blanking using the same DNase/RNase-Free Water used for elution.
11. Store eluted RNA at -80 ºC, and eluted DNA at -20 ºC.

**RNA Clean & Concentrator Tube Prep:**

Zymo-Spin IC Column w/ collection tube

1.5 mL catch tube