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

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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 vortex.
3. Add an equal volume (150 µL) of 100% ethanol and mix by vortex.
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, 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 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 at -80 ºC.

**RNA Clean & Concentrator Tube Prep:**

Zymo Spin Column w/ collection tube

1.5 mL catch tube