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

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

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

1. For each sample, label and UV-sterilize one set of 1.5 mL and 0.6 mL tubes.
2. Heat DNase/RNase-Free Water tube on a heat block at 55 °C.
3. Adjust sample volumes to 50 µLwith DNase/RNase-Free Water.
4. Add 2 volumes RNA Binding Buffer (100 µL) to each sample and mix by pipetting.
5. Add an equal volume of ethanol (150 µL) and mix by pipetting.
6. Transfer the sample to the Zymo-Spin column in a collection tube and centrifuge for 30 s. Discard the flow-through.
7. Add 400 µL RNA Prep Buffer to the column and centrifuge for 30 s. Discard the flow-through.
8. Add 700 µL RNA Wash Buffer to the column and centrifuge for 30 s. Discard the flow-through.
9. Add 400 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.Add 20 μL DNase/RNase-Free Water directly to the column matrix and centrifuge for 30 s.
10. Add 20 µL DAase/RNase-Free Water directly to the column matrix. Incubate at room temperature for 1–2 min. Centrifuge for 1 min.
11. Transfer purified RNA to a new 0.6 mL tube and store at -80 ºC.