**Zymo Quick-RNA MiniPrep Plus Extraction Protocol**

Written by M. Studivan

Updated: 11.16.2023 M. Studivan

This protocol assumes samples are preserved in 1000 µl Zymo DNA/RNA Shield.

All centrifugation steps are performed at **room temperature** and **15,000 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. Store at -20ºC until needed.

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

Optional: Prior to first use, add 3120 µl Proteinase K Storage Buffer to each Proteinase K (60 mg) bottle. The final concentration of Proteinase K is ~20 mg/ml. Store at -20ºC after mixing.

Optional: For freshly-collected samples, add 1000 µl of Zymo DNA/RNA Shield.

1. Thaw sample tubes and bead-beat for 2 min on the FastPrep (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.
2. Centrifuge for 2 min, then transfer 350 µL of homogenate to 2.0 mL tube. Re-freeze sample tube at -80ºC.
3. Optional: For freshly-collected samples, add 15 µL of Proteinase K and 30 µL of PK Digestion Buffer for every 300 µL of homogenate (17.5 µL ProK and 35 µL Buffer for 350 µL of homogenate). Vortex 10-15 sec and incubate at room temperature for 30 min. Centrifuge for 2 min, then transfer 350 µL of homogenate to new 2.0 mL tube.
4. Add equal volume of RNA Lysis Buffer (350 µL) and vortex 10-15 sec.
5. Transfer 700 µl of lysate to a Spin-Away Filter (**yellow**) in a collection tube. Centrifuge lysate for 30 s, and **save the flow-through**.
6. Add 1 volume ethanol (700 µL) to the flow-through and vortex 10-15 sec.
7. Transfer to a Zymo-Spin IICG Column (**green**) in a collection tube and centrifuge for 30 s. Discard flow-through, dab the collection tube on a KimWipe, then repeat for remaining 700 µL. Transfer spin column to a **new** **collection tube**.
8. 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.
9. 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.
10. Add 80 µL of DNase mix directly to each column.
11. Incubate at room temperature for 25 min.
12. Add 400 µL RNA Prep Buffer to the column and centrifuge for 30 s. Discard the flow-through and dab collection tube on a KimWipe.
13. Add 700 µL RNA Wash Buffer and invert **only the column** twice. Centrifuge for 30 s, pour out the flow-through, and dab the collection tube on a KimWipe.
14. Add 400 µL RNA Wash Buffer to the filter directly (**do not invert**) and centrifuge for 1 min. Carefully transfer spin column to a new 1.5 mL tube. Avoid contact of flow through and spin column tip.
15. To elute RNA, add 50 µL of nuclease-free water heated to 60 ºC directly to the column, incubate for 5 min, and centrifuge at 19,000 x *g* for 1 min.
16. Store eluted RNA **on ice** until the next kit protocol.

**MiniPrep Plus Tube Prep:**

2.0 mL tube for homogenate

Spin-Away Filter (**yellow**) w/ collection tube

Zymo-Spin IICG Column (**green**) w/ collection tube

New collection tube

1.5 mL catch tube

**Zymo OneStep PCR Inhibitor Removal Protocol**

Written by M. Studivan

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1. 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.
2. Transfer 50 µL of eluted DNA/RNA into prepared Zymo-Spin III-HRC Filter and centrifuge at 16,000 x *g* for 3 min.
3. Store eluted RNA **on ice** until the next kit protocol.

**HRC Tube Prep:**

Zymo-Spin III-HRC Filter w/ collection tube

1.5 mL catch tube

**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 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. Store eluted RNA **on ice** until long-term storage.
10. Nanodrop eluted RNA, blanking using the same DNase/RNase-Free Water used for elution.
11. Store eluted RNA at -80 ºC.

**RNA Clean & Concentrator Tube Prep:**

Zymo-Spin IC Column w/ collection tube

1.5 mL catch tube