RNA Extraction Protocols for iSWAB-RNA v2

Kit: Zymo Quick-RNA™ MiniPrep Plus (**Cat. Nos. R1057 and R1058**) – Highly Recommended

Whole Blood (mammalian) Protocol – Do not use DNase treatment

1. Add 300 µl DNA/RNA Shield™ (2X concentrate) directly to pelleted cells from **iSWAB-RNA v2*** and mix thoroughly.
   - **Note:** Tip mix 5-10 times gently of the collected sample in iSWAB- before adding it DNA/RNA shield buffer)

   - ***Note**: Let the **iSWAB-RNA v2** collected sample set overnight at room temperature, then remove the supernatant and add 300 µl DNA/RNA Shield™ (2X concentrate) directly to the pelleted buccal cells. After mixing thoroughly, remove to content of the mixture to a clean Rnase free Eppendorf tube before proceeding to step 2

2. Add 12 µl Proteinase K and mix thoroughly.

3. Incubate at room temperature (20-30º C) for 30 - 60 minutes.

4. Add an equal volume of isopropanol and mix by vortex. Proceed to page 6, step 3 as described in the kit protocol.
   - **Note:** The total volume will be a total of 1200 µl, which is higher than the capacity of the green column.

5. Transfer 600 µl of the mixture to a Zymo-Spin™ IIICG Column (**green**) in a Collection Tube and centrifuge. Discard the flow-through. Apply the reminder 600 µl to the column and centrifuge again. Discard the flow-through and continue to step 6.
   - **Note:** All centrifugation steps should be performed at 10,000 – 16,000 x g for 30 seconds unless specified**.**

6. Add 400 µl RNA Prep Buffer to the column and centrifuge. Discard the flowthrough.

7. Add 700 µl RNA Wash Buffer to the column and centrifuge. Discard the flowthrough

8. Add 400 µl RNA Wash Buffer and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).

9. Add 50 µl DNase/RNase-Free Water directly to the column matrix and centrifuge.

10. Repeat step 9 by applying the eluent volume back to the column to ensure release of the majority of the RNA and concentrate the RNA.

11. The eluted RNA can be used immediately or stored at ≤-70º C.

Kit: QIAamp RNA Blood (**Cat. No. 52304**)

Start on Page 19, Step 6 – Do not use DNase treatment

1. In RNase/DNase free 1.5 ml Eppendorf tube, add Buffer 350 µl from RLT* to **ISWAB-RNA v2*** pelleted cells. Then add 20 µl from proteinase K stock (20 mg/mL) (Not provided with the kit). Mix well by vortexing and Incubate at room temperature (20-30ºC) for 30 - 60 minutes.
   - **Note:** No cell clumps should be visible before you proceed to the homogenization step. Vortex or pipet further to remove any clumps. * Ensure β-ME is added to Buffer RLT (see "Things to do before starting").
   - ***Note**: Let the **iSWAB-RNA v2** collected sample set overnight at room temerpature, then remove the supernatant and add 350 µl from RLT directly to the pelleted buccal cells. After mixing thoughly, remove to content of the mixture to a clean Rnase free eppendorf tube. Then add 20 µl from proteinase K stock (20 mg/mL) (Not provided with the kit). Mix well by vortexing and Incubate at room temperature (20-30ºC) for 30 - 60 minutes. before Proceed to step 2

2. Pipet lysate directly into a QIAshredder spin column in a 2 ml collection tube (provided) and centrifuge for 2 min at maximum speed to homogenize. Discard QIAshredder spin column and save homogenized lysate. To avoid aerosol formation, adjust pipet to 750 µl to ensure that the lysate can be added to the QIAshredder spin column in a single step.

3. Add 1 volume (750 µl) of 70% ethanol to the homogenized lysate and mix by pipetting. Do not centrifuge. A precipitate may form after the addition of ethanol. This will not affect the QIAamp procedure.

4. Carefully pipet sample, including any precipitate which may have formed, into a new QIAamp spin column in a 2 ml collection tube (provided) without moistening the rim. Centrifuge for 15 s at 8000 x g (10,000 rpm). Maximum loading volume is 700 µl. Since the volume of the sample exceeds 700 µl, successively load aliquots onto the QIAamp spin column and centrifuge as above. Discard flow-through* and collection tube.

5. Transfer the QIAamp spin column into a new 2 ml collection tube (provided). Apply 700 µl Buffer RW1 to the QIAamp spin column and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash. Discard flow-through* and collection tube.

6. Place QIAamp spin column in a new 2 ml collection tube (provided). Pipet 500 µl of Buffer RPE into the QIAamp spin column and centrifuge for 15 s at 8000 x g (10,000 rpm). Discard flow-through* and collection tube.
   - **Note:** Ensure ethanol is added to Buffer RPE (see ”Things to do before starting”).

7. Carefully open the QIAamp spin column and add 500 µl of Buffer RPE. Close the cap and centrifuge at full speed (20,000 x g, 14,000 rpm) for 3 min.
   - **Note:** Some centrifuge rotors may distort slightly upon deceleration, resulting in flow-through, containing Buffer RPE, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column

8. Recommended: Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. This step helps to eliminate the chance of possible Buffer RPE carryover.

9. Transfer QIAamp spin column into a 1.5 ml microcentrifuge tube (provided) and pipet 50 µl of RNase-free water (provided) directly onto the QIAamp membrane. Centrifuge for 1 min at 8000 x g (10,000 rpm) to elute.

10. Repeat step 9 by applying the eluent volume back to the column to ensure release of the majority of the RNA and concentrate the RNA.

11. The eluted RNA can be used immediately or stored at ≤ -70º C.