QIAGEN Supplementary Protocol:

Simultaneous Purification of DNA and RNA from Saliva Stabilized in RNAprotect® Saliva Reagent

This protocol describes how to purify DNA and RNA from human saliva samples stabilized in RNAprotect Saliva Reagent (the reagent provides immediate stabilization of both DNA and RNA at room temperature). The purification procedure requires use of both the QIAamp® DNA Mini Kit and the RNeasy® Protect Saliva Mini Kit. Separate eluates of DNA and RNA are simultaneously purified from the same sample.

IMPORTANT: Please read the handbooks supplied with the RNeasy Protect Saliva Mini Kit and QIAamp DNA Mini Kit, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- RNeasy Protect Saliva Mini Kit (cat. no. 74324)
- QIAamp DNA Mini Kit (cat. no. 51304 for 50 preps; cat. no. 51306 for 250 preps)
- Ethanol (96–100%) (do not use denatured alcohol, which contains other substances such as methanol or methylethylketone)
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Vortexer
- Heating block or water bath capable of reaching 56°C

Important points before starting

- If working with RNA for the first time, read Appendix A in the *RNeasy Protect Saliva Mini Handbook*.
- Saliva samples generally contain very low amounts of RNA. To ensure maximal RNA yields as well as maximal DNA yields, store the stabilized saliva sample for at least 24 h before starting RNA and DNA purification. Due to the heterogenous nature of saliva, shorter storage times may be sufficient for some samples. However, we still recommend storage for at least 24 h.
- Buffer RLT, Buffer RW1, and Buffer AW1 contain a guanidine salt, and are therefore not compatible with disinfecting reagents containing bleach. For safety information, see the handbooks supplied with the RNeasy Protect Saliva Mini Kit and QIAamp DNA Mini Kit.
- Buffer ATL and Buffer AL are not needed in this protocol.
- Vortexing should be carried out by pulse-vortexing for 5–10 s.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all spin column centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- Stabilize and store saliva in RNAprotect Saliva Reagent as described in the RNA stabilization protocol in the *RNeasy Protect Saliva Mini Handbook*.
- Before using the RNeasy Protect Saliva Mini Kit for the first time, prepare 80% ethanol by mixing 24 ml ethanol (96–100%) with 6 ml RNase-free water (supplied with the kit).
- Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).
- Buffer RPE, Buffer AW1, and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. **Do not vortex**.

  For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.
- Preheat a heating block or water bath to 56°C for use in step 19.

Procedure

1. **Centrifuge the mix of saliva and RNAprotect Saliva Reagent for 10 min at 10,000 x *g* in a microcentrifuge.**

   Note: The stabilized saliva sample must be stored for at least 24 h prior to centrifugation (see "Important points before starting").

   Note: If the sample was stored below room temperature (e.g., 2–8°C or –20°C), thaw it completely and equilibrate it to room temperature before starting centrifugation.

   Note: A precipitate may form during storage, especially at lower temperatures. This does not affect nucleic acid purification.

2. **Remove the supernatant completely by pipetting.**

3. **Loosen the pellet by flicking the tube.**

   Loosening the pellet facilitates dissolving in Buffer RLT in step 4.

4. **Add 350 µl Buffer RLT. Dissolve the pellet completely by vortexing.**

   Note: Be sure to dissolve the pellet completely. This can take about 1 min.

   Note: The dissolved pellet may be turbid. This does not affect nucleic acid purification.

   The dissolved pellet can be stored at –70°C for several months. After removal from storage, incubate the dissolved pellet at room temperature or at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation at 37°C, which can cause RNA degradation. Proceed to step 5.

5. **Transfer the lysate to a QIAamp Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm).**

   Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

6. **Place the QIAamp Mini spin column in a new 2 ml collection tube (supplied), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 17–23. Use the flow-through for RNA purification in steps 7–16.**

   Note: Do not store the QIAamp Mini spin column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA purification

7. **Add 1 volume (350 µl) of 70% ethanol, and mix well by pipetting or vortexing. Do not centrifuge. Proceed immediately to step 8.**

   A precipitate may form after addition of ethanol, but this does not affect the procedure.

8. **Transfer the sample to an RNeasy MinElute® spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

   Reuse the collection tube in step 9.

9. **Add 350 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.***

   Reuse the collection tube in step 10.

10. **Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube.**

    Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.

11. **Add the DNase I incubation mix (80 µl) directly to the RNeasy MinElute spin column membrane, and incubate on the benchtop (20–30°C) for 15 min.**

    Note: Be sure to add the DNase I incubation mix directly to the RNeasy MinElute spin column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

12. **Add 350 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through* and collection tube.**

13. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Add 500 µl Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

    Reuse the collection tube in step 14.

    Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

14. **Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and collection tube.**

    Prepare the 80% ethanol using ethanol (96–100%) and the RNase-free water supplied with the kit.

    Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

* Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See the *RNeasy Protect Saliva Mini Handbook* for safety information.

15. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.**

    To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

16. **Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.**

    The dead volume of the RNeasy MinElute spin column is 2 µl; elution with 14 µl RNase-free water results in a 12 µl eluate.

    Elution with smaller volumes of RNase-free water leads to higher total RNA concentrations, but lower RNA yields. RNA yield will be reduced by approximately 20% if RNA is eluted in 10 µl RNase-free water. We do not recommend eluting RNA in less than 10 µl RNase-free water, as the spin column membrane may not be sufficiently hydrated.

Genomic DNA purification

17. **Add 350 µl Buffer AW1 to the QIAamp Mini spin column from step 6. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flow-through.***

    Reuse the collection tube in step 20.

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Important points before starting").

18. **Add 20 µl QIAGEN Proteinase K to 60 µl distilled water. Mix by vortexing.**

19. **Add the proteinase K incubation mix (80 µl) directly to the QIAamp Mini spin column membrane, and incubate at 56°C for 10 min.**

    Note: Be sure to add the proteinase K incubation mix directly to the QIAamp Mini spin column membrane. Proteinase K digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

20. **Add 350 µl Buffer AW1 to the QIAamp Mini spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through* and collection tube.**

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See the handbook supplied with the QIAamp DNA Mini Kit for safety information.

21. **Place the QIAamp Mini spin column in a new 2 ml collection tube (supplied), and add 500 µl Buffer AW2. Close the lid gently, and centrifuge for 3 min at full speed to wash the spin column membrane.**

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Important points before starting").

    The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during DNA elution. Residual ethanol may interfere with downstream reactions.

    Note: After centrifugation, carefully remove the QIAamp Mini spin column from the collection tube. If the column contacts the flow-through, empty the collection tube and centrifuge the spin column again for 1 min at full speed.

22. **Place the QIAamp Mini spin column in a new 1.5 ml or 2 ml microcentrifuge tube (not supplied). Add 100 µl Buffer AE or distilled water directly to the spin column membrane, and close the lid. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.**

23. **Repeat step 22 to elute further DNA.**

    To prevent dilution of the first DNA eluate, use a new 1.5 ml or 2 ml microcentrifuge tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the tube from step 22.

    Note: To achieve a higher DNA concentration, elute with 2 x 50 µl Buffer AE or distilled water. The final DNA yield, however, may be reduced.