QIAGEN Supplementary Protocol:

Purification of DNA from Saliva Stabilized in RNAprotect® Saliva Reagent

This protocol describes how to purify DNA 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 the QIAamp® DNA Mini Kit only.

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.

- For stabilization of saliva samples: 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)
- PBS (50 mM potassium phosphate, 150 mM NaCl, pH 7.2)
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Vortexer
- Thermomixer, shaking water bath, or rocking platform capable of reaching 56°C
- Optional: RNase A (100 mg/ml) (cat. no. 19101)
- Optional: Buffer AL (cat. no. 19075) and QIAGEN® Proteinase K (cat. no. 19131 for 2 ml; cat. no. 19133 for 10 ml) (both are supplied with the QIAamp DNA Mini Kit, but additional quantities can be purchased separately if necessary)

Important points before starting

- To ensure maximal DNA yields, store the stabilized saliva sample for at least 24 h before starting 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 AL and Buffer AW1 contain a guanidine salt, and are therefore not compatible with disinfecting reagents containing bleach. For safety information, see the handbook supplied with the QIAamp DNA Mini Kit.
- 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.
- Vortexing should be carried out by pulse-vortexing for 5–10 s.
- Buffer ATL is not required in this protocol.
- Optional: RNA can be digested during the procedure using RNase A (not supplied with the QIAamp DNA Mini Kit). RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

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*.
- Buffer AL may form a precipitate during storage. If necessary, warm to 56°C until fully dissolved.
- 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.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 6.

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 DNA purification.

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

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

   Loosening the pellet facilitates dissolving in PBS in step 4.

4. **Add 1 ml PBS. Dissolve the pellet by vortexing.**

5. **Centrifuge for 5 min at 1800 x *g*, and carefully remove the supernatant. Resuspend the pellet in 180 µl PBS.**

   Optional: If RNA-free genomic DNA is required, add 5 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature before continuing with step 6.

6. **Add 25 µl QIAGEN Proteinase K and 200 µl Buffer AL. Mix immediately and thoroughly by vortexing, and incubate at 56°C for 10 min.**

   Note: Do not add QIAGEN Proteinase K directly to Buffer AL.

   Note: Immediate and thorough mixing is essential to yield a homogenous solution. Do not add ethanol to Buffer AL.

   Note: The speed of the thermomixer should be set to 900 rpm. If mixing during incubation is not possible, the sample can be vortexed once or twice instead during the 10-min incubation.

   Note: Since this protocol uses a larger amount of QIAGEN Proteinase K compared with standard QIAamp DNA Mini procedures, additional QIAGEN Proteinase K may need to be purchased if the QIAamp DNA Mini Kit will be used mainly for saliva samples.

7. **Add 200 µl ethanol (96–100%), and mix thoroughly by vortexing**

   Note: Thorough mixing is essential to yield a homogenous solution.

8. **Transfer the sample, including any precipitate formed, to a QIAamp Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard the flow-through* and collection tube.**

9. **Place the QIAamp Mini spin column in a new 2 ml collection tube (supplied), and add 500 µl Buffer AW1. Close the lid gently, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to wash the spin column membrane. Discard the flow-through* and collection tube.**

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

10. **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. Discard the flow-through and collection tube.**

    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.

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

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.

11. **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 ≥6000 x *g* (8000 rpm) to elute the DNA.**

12. **Repeat step 11 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 11.

    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.