OMNIgene™•SALIVA
DNA and RNA

The purification of human RNA from OMNIgene™•SALIVA DNA and RNA (OMR-610) samples using the QIAGEN RNeasy® Micro Kit and QIAGEN RNeasy® Mini Kit

This laboratory protocol is used for the preparation of a saliva sample collected and stabilized in OMNIgene™•SALIVA DNA and RNA (OMR-610) devices for the subsequent extraction of human RNA using an RNeasy Micro Kit (QIAGEN, Cat. No. 74004) or an RNeasy Mini Kit (QIAGEN, Cat. No. 74104).

Equipment and reagents

- RNeasy Micro Kit (QIAGEN, Cat. No. 74004), instructions and components, including Buffer RLT, MinElute spin column, collection tubes, Buffer RW1, DNase I stock solution, Buffer RDD, Buffer RPE and RNase-free water.
    - Alternatively, the RNeasy Mini Kit (QIAGEN, Cat. No. 74104) can be used in combination with the RNase-Free DNase Set (QIAGEN, Cat. No. 79254).
- Triton X-100 (e.g., 100 mL of MelliporeSigma 9400 OmniPur), Tween-20 (e.g., Sigma-Aldrich P7949) or Tween-80 (e.g., VWR BDH7781-2).
- Ethanol solutions: 80% (at room temperature) and 95% (at room temperature).

Procedure

Sample preparation steps
1. Incubate the saliva samples: a. Shake sample vigorously for 10 seconds. b. Incubate the entire sample in the original collection tube at 50°C for 1 hour in a water bath or for 2 hours in an air incubator. (This step does not need to be repeated for the purification of RNA from subsequent aliquots of the sample.) **Note:** To increase RNA yields, double load the column by processing two 250 µL aliquots of an OMR-610 sample and repeating step 5 with the second aliquot.
2. Prepare a modified Buffer RLT with 5% (vol/vol) Triton X-100 or Tween-20, or, alternatively, 2% (vol/vol) Tween-80. **Example:** For each 250 µL OMR-610 extraction aliquot, prepare at least 260 µL of modified Buffer RLT by adding 13 µL Tween-20 or Triton X-100, or add 5.2 µL Tween-80 to 260 µL of Buffer RLT.
3. Transfer 250 µL of the OMR-610 sample to a 1.5 mL microcentrifuge tube.
4. To the 250 µL OMR-610 aliquot, add 250 µL of the modified RNeasy Buffer RLT and 250 µL of the 95% ethanol. Invert the sample 6x to mix.
5. Transfer the combined sample/RLT/ethanol mixture onto an RNeasy MinElute spin column in a 2 mL collection tube. Close the lid and centrifuge for 15 seconds at > 8,000 × *g*. Discard the flow-through. If double-loading the column to increase RNA yield, repeat this step with the second sample aliquot. Reuse the collection tube in step 6.
6. Add 350 µL of Buffer RW1 to the RNeasy MinElute spin column. Close the lid and centrifuge for 15 seconds at > 8,000 × *g*. Discard the flow-through. Reuse the collection tube in step 8.
7. Add 10 µL DNase I stock solution to 70 µL Buffer RDD. Invert the tube gently to mix the sample.
8. Add the 80 µL DNase I incubation mix directly onto the RNeasy MinElute spin column membrane and incubate on the benchtop for 15 minutes.

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Sample preparation steps
9. Add 350 µL Buffer RW1 to the RNeasy MinElute spin column. Close the lid and centrifuge for 15 seconds at > 8,000 × *g*. Discard the flow-through and collection tube.
10. Place the RNeasy MinElute spin column into a fresh 2 mL collection tube. Add 500 µL Buffer RPE to the spin column. Close the lid and centrifuge for 15 seconds at > 8,000 × *g*. Discard the flow-through. Reuse the collection tube in step 11.
11. Add 500 µL of 80% ethanol to the RNeasy MinElute spin column. Close the lid and centrifuge for 2 minutes at > 8,000 × *g*. Discard the flow-through and collection tube.
12. Place the RNeasy MinElute spin column into a fresh 2 mL collection tube. Open the lid of the spin column and centrifuge at ≥ 16,000 × *g* for 5 minutes. Discard the flow-through and collection tube.
13. Place the RNeasy MinElute spin column into a fresh 1.5 mL collection tube. Add 50 µL of RNase-free water directly to the center of the spin column membrane. Incubate at room temperature for 5 minutes. Close the lid and centrifuge for 1 minute at ≥ 16,000 × *g* to elute the RNA. **Note:** If you require higher RNA concentrations and you are using an RNeasy Micro Kit (QIAGEN, Cat. No. 74004), you may reduce the elution volume to 25 µL.

Technical support is available Monday to Friday (9:00 a.m.-5:00 p.m. ET):

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