ORAgene®·RNA ORAcollect®·RNA

RNA from saliva purification protocol for volumes up to 250 µL

(To purify more than 250 µL you may follow the protocol described in PD-PR-021)

Equipment and reagents

- Neutralizer solution (Cat. No. RE-LZN, purchased separately)
- Ethanol solutions: 70% and 80% (room temp), 95% (-20°C)
- Qiagen RNeasy Micro Kit (Cat. No. 74004) and instructions. Components of the RNeasy kit: RLT buffer, MinElute spin column, collection tubes, RW1 buffer, DNase I stock solution, RDD buffer, RPE buffer and RNase-free water. Alternatively, the Qiagen RNeasy Mini Kit (Cat. No. 74104) can be used in combination with the Qiagen RNase-Free DNase Set (Cat. No. 79254)

Steps prior to purification of RNA from saliva

1. When samples are received in the lab, shake very vigorously for 8 seconds or longer.
2. Samples may be stored at room temperature for up to 8 weeks or stored frozen at -20°C indefinitely.
3. Prior to purification, incubate entire sample in original collection tube at 50°C for 1 hour in a water bath or for 2 hours in an air incubator.

Purification preparation steps

1. Remove a 250 µL aliquot to a 1.5 mL microcentrifuge tube.
2. Incubate this aliquot at 90°C for 15 minutes, then cool to room temperature.
3. Add 1/25th volume of neutralizer solution. (e.g., 10 µL neutralizer for a 250 µL sample). Incubate on ice for 10 minutes.
4. Centrifuge at maximum speed (> 13,000 × *g*) for 3 minutes.
5. Taking care not to disturb the pellet, carefully remove supernatant to a fresh tube; discard the pellet. The supernatant is neutralized.
6. Prepare Qiagen RNeasy solution by combining 250 µL of RNeasy RLT buffer with 250 µL of 95% ethanol.
7. To this RLT/ethanol mixture, add up to 250 µL of the supernatant; mix 6× by gentle inversion.
8. Proceed immediately to the RNeasy purification instructions.

For Laboratory Use Only

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Qiagen RNeasy purification procedure

Start at step #5 of the Qiagen RNeasy Micro Kit "Total RNA isolation from animal cells" protocol. The following brief version of the protocol is provided for your convenience. (Note slight modification to the elution step #13).

5. Transfer the sample 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. 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. Mix by gently inverting the tube.
8. Add the DNase I incubation mix (80 µL) directly onto the RNeasy MinElute spin column membrane and incubate on the benchtop for 15 minutes.
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 full speed 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 25 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 full speed to elute the RNA.

Technical support is available Monday to Friday (9h00 to 17h00 ET):

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