ORAgene®•RNA

Oragene®•RNA purification protocol using the Qiagen RNeasy Micro Kit for volumes up to 1,000 µL

(To purify less than 250 µL you may follow the protocol described in PD-PR-028)

Equipment and reagents

- Oragene®•RNA neutralizer solution (supplied with Oragene•RNA kit)
- Ethanol solutions: 70% and 80% (room temperature), 95% (-20°C)
- Qiagen RNeasy Micro Kit (Cat. No. 74004) and instructions. Components required from the RNeasy kit are RLT buffer and 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).

Part I – Laboratory preparation and storage of a saliva/Oragene•RNA sample

Purification steps	Notes
1. When samples are received in the lab, shake very vigorously for 8 seconds or longer.	• Thorough mixing of the Oragene•RNA solution and saliva is necessary to ensure maximum RNA recovery and stability.
2. Incubate entire sample in original vial at 50°C for 1 hour in a water bath or for 2 hours in an air incubator.	• Entire sample must be heated at 50°C prior to any subsequent purification. • Samples may be stored at room temperature for up to 8 weeks or stored frozen at -20°C indefinitely before or after the heating step.

Part II – Purification of an aliquot of saliva/Oragene•RNA sample

Purification steps	Notes
1. Transfer a 250 – 500 µL aliquot to a 1.5 mL microcentrifuge tube.	• 1,000 µL aliquot should be processed in 2 tubes • Any remaining material in the Oragene•RNA container can be stored for up to 8 weeks at room temperature or stored frozen at -20°C indefinitely.
2. Incubate the aliquot at 90°C for 15 minutes, then cool to room temperature.	• Care should be taken not to exceed 90°C to ensure the sample does not degrade. A water bath incubator is the preferred method of heating. A heating block can be used but should be monitored closely.
3. Add 1/25th volume of neutralizer solution. Vortex to mix thoroughly.	
4. Incubate on ice for 10 minutes.	• The sample will become turbid as impurities and inhibitors are precipitated.

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Not for use in diagnostic procedures

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Purification steps	Notes
5. Centrifuge in microcentrifuge at maximum speed (> 13,000 × *g*) for 3 minutes.	
6. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. Discard the pellet containing impurities.	• The pellet contains turbid impurities. If accidentally disturbed, the tube should be re-centrifuged to remove impurities.
7. Add 2 volumes of cold 95% EtOH to the clear supernatant. Mix thoroughly by inversion, vortexing or shaking.	• e.g., for a 250 µL aliquot use 500 µL of 95% EtOH; for a 500 µL aliquot use 1000 µL of 95% EtOH. • Mixing with ethanol precipitates the nucleic acids.
8. Incubate at -20°C for 30 minutes.	• Incubation at -20°C is necessary to ensure maximum precipitation of RNA.
9. Place the tube into the microcentrifuge in a known orientation. Collect precipitate by centrifugation at maximum speed (> 13,000 × *g*) for 3 minutes.	• For example, place each tube in the microcentrifuge with the hinge portion of the cap pointing away from the centre of the rotor. After centrifugation, the position of the pellet can be located (even if very tiny); it will be on the side of the tube near the tip below the hinge.
10. Carefully remove and discard the supernatant, taking care to avoid disturbing the pellet.	• This pellet contains the purified nucleic acids. Loss of the pellet will result in loss of the RNA. • Rotating the tube such that the pellet is on the upper wall will allow you to safely move a pipette tip along the lower wall and remove all of the supernatant.
11. Dissolve the pellet in 350 µL of buffer RLT (RNeasy Micro Kit) by vigorous vortexing, taking care to ensure that the pellet is completely dissolved.	• Vigorous vortexing may be required to dissolve the pellet. • Pipetting up and down several times can help to break apart and dissolve the pellet. • May take a few minutes for the pellet to be completely dissolved.
12. Add 350 µL of 70% ethanol. Mix well by vortexing.	• It is normal to see small particulate matter.
13. Proceed immediately to the Qiagen RNeasy Cleanup instructions.	

Part III – Qiagen RNeasy cleanup 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. Re-use 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. Re-use the collection tube in step 8.
7.	In a separate tube 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 at room temperature 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. Re-use 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 EST):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: 613.723.5757, option 6
- Email: support@dnagenotek.com