ORAcollect™•RNA

Extraction of RNA from an ORAcollect™•RNA device (OR-100, ORE-100) using the QIAGEN® miRNeasy Mini kit

Required equipment and reagents:

- miRNeasy Mini kit (QIAGEN®, Cat. No. 217004)
- Microcentrifuge capable of running at 12,000 x *g*
- Microcentrifuge tubes
- Air incubator or water bath at 50°C
- Microtube heating block or water bath at 90°C
- Ethanol (96%-100%)
- Chloroform

Procedure

Part I — Steps prior to purification of RNA from saliva

Procedure	Notes
1. Shake sample to thoroughly mix	
2. Prior to extraction, incubate the entire sample in the original collection tube at 50°C for 1 hour in a water bath or 2 hours in an air incubator.	Following incubation, samples may be stored at room temperature for a maximum of 8 weeks from the date of sample collection, or stored frozen at -20°C.

Part II — Laboratory preparation and storage of ORAcollect™•RNA sample

Procedure	Notes
1. Transfer a 250 µL aliquot of the incubated ORAcollect™•RNA sample to a 1.5 mL microcentrifuge tube.	Any remaining sample in the ORAcollect™•RNA tube can be stored at room temperature for a maximum of 8 weeks from the date of sample collection, or stored frozen at -20°C.
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. This heating step inactivates RNase enzymes.
3. Add 750 µL QIAzol Lysis Reagent (QIAGEN). Vortex for 1 minute.	The QIAzol Lysis Reagent (QIAGEN) also inactivates RNase enzymes due to the presence of phenol.
4. Place the tube containing the homogenate on the benchtop at room temperature (15°C–25°C) for 5 minutes.	This step promotes the dissociation of nucleoprotein complexes.
5. Add 200 µL chloroform to the tube containing the homogenate and cap it securely. Vortex for 15 seconds.	Thoroughly mixing the chloroform is important for the subsequent phase separation.

Procedure	Notes
6. Place the tube containing the homogenate on the benchtop at room temperature for 2–3 minutes.	
7. Centrifuge for 15 minutes at 12,000 x *g*.	Centrifugation will cause the sample to separate into 3 phases: an upper-colorless aqueous phase containing RNA, a white interphase and a lower-red organic phase.
8. Transfer 450 µL of the upper aqueous phase to a new collection tube.*	Use caution not to disturb the lower phases.
9. Add 675 µL of ethanol and mix thoroughly by pipetting up and down several times. Do not centrifuge. Continue without delay to the next step.	A precipitate may form after the addition of ethanol, but will not affect the procedure.
10. Transfer up to 700 µL of the sample, including any precipitate that may have formed, into a spin column in a supplied 2 mL collection tube. Close the lid gently and centrifuge at ≥ 8,000 x *g* (≥ 10,000 rpm) for 15 seconds at room temperature (15°C–25°C). Discard the flow-through. Reuse the collection tube.*	
11. Repeat the previous step using the remainder of the sample. Discard the flow-through. Reuse the collection tube.*	
12. Add 700 µL Buffer RWT (QIAGEN) to the miRNeasy Mini (QIAGEN) spin column. Close the lid gently and centrifuge for 15 seconds at ≥ 8,000 x *g* (≥ 10,000 rpm) to wash the column. Discard the flow-through. Reuse the collection tube.*	
13. Pipette 500 µL Buffer RPE (QIAGEN) onto the miRNeasy Mini (QIAGEN) spin column. Close the lid gently and centrifuge for 15 seconds at ≥ 8,000 x *g* (≥ 10,000 rpm) to wash the column. Discard the flow-through. Reuse the collection tube.*	

Procedure	Notes
14. Add another 500 µL Buffer RPE (QIAGEN) onto the miRNeasy Mini (QIAGEN) spin column. Close the lid gently and centrifuge for 2 minutes at ≥ 8,000 x *g* (≥ 10,000 rpm) to dry the miRNeasy Mini (QIAGEN) spin column membrane.	The long centrifugation dries the spin column membrane, ensuring no ethanol is carried over during RNA elution. Use caution when removing the miRNeasy Mini (QIAGEN) spin column from the collection tube; ensure the spin column does not contact the flow-through. Residual ethanol or ethanol carried over may interfere with downstream assays.
15. Transfer the miRNeasy (QIAGEN) Mini spin column to a new 1.5 mL collection tube. Pipette 30 µL RNase-free water directly onto the miRNeasy Mini (QIAGEN) spin column membrane. Close the lid gently and centrifuge for 1 minute at ≥ 8,000 x *g* (≥ 10,000 rpm) to elute the RNA. Reuse the collection tube.	
16. Pipette a second volume of 30 µL RNase-free water directly onto the miRNeasy Mini (QIAGEN) spin column membrane. Close the lid gently and centrifuge for 1 minute at ≥ 8,000 x *g* (≥ 10,000 rpm) to elute RNA. Place purified RNA on ice or store at -20°C.	This step elutes RNA remaining in the column to increase the total RNA yield.

* Flow-through contains guanidine and is, therefore, not compatible with bleach.

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