Total RNA extraction from saliva samples collected with Oragene®•RNA (Genotek) (Rev. 02)

This support protocol describes the RNA isolation from saliva which has been collected in Oragene®•RNA (Cat.No. RE-100; DNA Genotek Inc., Canada).
When samples are received in the lab, shake them vigorously for 8 seconds or longer. Thorough mixing of the Oragene®•RNA solution and the saliva is necessary to ensure maximum RNA recovery and stability.

Before starting the preparation, heat a water bath to 50°C and 90°C for step 1 and step 3, respectively. Check if Wash Buffer RA3 and rDNase were prepared according to the NucleoSpin® RNA II user manual.

Procedure

1. Incubate entire sample in Oragene®•RNA vial at **50°C** for **1 h** in a water bath.

Note: *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.*

2. Transfer **250 µL of sample** into a 1.5 microcentrifuge tube.

3. Incubate at **90°C** for **15 min**. Let cool down to room temperature (18-25°C).

4. Add **1/25 volume (10 µL)** of **Oragene®•RNA Neutralizer Solution** (supplied with Oragene®•RNA kit). Vortex to mix thoroughly.

5. Incubate **on ice** for **10 min**.

6. Centrifuge in microcentrifuge at maximum speed (**>13,000 × *g***) for **3 min**.

7. Carefully transfer the clear supernatant into a fresh microcentrifuge tube. Discard the pellet containing impurities.

8. Add **250 µL Buffer RA1** and **3.5 µl β-mercaptoethanol** and mix.

9. Add **250 µL ethanol (96%)**, mix, and spin down briefly.

10. Continue with **step 6** (Bind RNA) of the **standard protocol**:
Load the sample to the NucleoSpin® RNA II Column.

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