

## **Support protocol NucleoSpin® RNA II: Total RNA extraction from saliva samples collected with Oragene®•RNA (Genotek)**

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 A) and step C), respectively.
- Check if Wash Buffer RA3 and rDNase were prepared according to section 3 of the NucleoSpin® RNA II User Manual.

**A)** 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.*

**B)** Transfer **250 µl of sample** into a 1.5 microcentrifuge tube.

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

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

**E)** Incubate **on ice** for **10 min**.

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

**G)** Carefully transfer the clear supernatant into a fresh microcentrifuge tube. Discard the pellet containing impurities.

**H)** Add **250 µl Buffer RA1** and **3.5 µl β-mercaptoethanol** and mix.

**I)** Add **250 µl 96% ethanol**, mix, and spin down briefly.

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

### **Trademarks:**

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NucleoSpin is a trademark of MACHEREY-NAGEL GmbH & Co. KG