NucleoSpin® RNA – RNA from saliva samples collected with Oragene®·RNA (Genotek) (Rev. 01)

This protocol is only a supplement to the kit's general user manual. Please refer to the kit manual for more detailed information regarding safety instructions, product-specific disclaimers, and especially preparations needed before starting the procedure. The latest version of the user manual is available at ***www.mn-net.com/usermanuals*** *or can be requested from our technical service (tech-bio@mn-net.com). Material safety data sheets (MSDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

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 that Wash Buffer RA3 and rDNase were prepared according to the NucleoSpin® RNA user manual.

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 the sample** into a 1.5 mL 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 (96–100 %)**, mix, and spin down briefly.

Proceed with step 5 of the standard protocol ('Bind RNA'). Load the sample to the NucleoSpin® RNA Column.