Supplementary protocol

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NucleoSpin® Tissue – isolation of genomic DNA from semen (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****.*

For an optimal performance, a differential lysis of different cell types is necessary, such as the separation of sperm DNA from epithelial cells and / or blood.

Additional equipment needed:

- The use of buffer GuEX (200 mL) is required. Buffer GuEX is prepared from 2 mL of a sterile 5 M Guanidine hydrochloride solution (should not be autoclaved), 2.1 mL of a 1 M Tris-Cl (pH 8) solution, 1.05 mL of a 2 M NaCl solution, 4.2 mL of 0.5 M EDTA solution, and 0.2 mL of a 1 M NaOH solution. Add water to a volume of 200 mL. The pH should be between 8–8.5.
- Isopropanol

Additional preparations before starting:

- Prepare buffer GuEX.

1 Transfer the sample in a 1.5 mL microcentrifuge tube. Add **950 µL buffer GuEX** and **50 µL Proteinase K** solution.

Incubate the mixture not longer than **15 min** at **37 °C**.

2 Centrifuge the mixture for **4 min** at **12,000 x *g*** at **room temperature**. The pellet contains sperm cells (sample A pellet) whereas the free DNA (from epithelial cells and leukocytes, sample B supernatant) is in the supernatant.

3 The supernatant (sample B supernatant) is removed carefully, transferred to a fresh tube, and processed separately (see step 6).

4 Add **700 µL buffer GuEX** to the pellet (sample A pellet), centrifuge for **4 min** at **12,000 x *g*,** and discard the supernatant. Repeat this wash step 2–3 times.

5 The pellet (sample A pellet) is resuspended in a minimum of **300 µL Buffer T1**.

6 Sample A pellet: Add **25 µL Proteinase K** stock solution, mix by vortexing, and incubate at **60–65 °C overnight**.

Sample B supernatant: Add **10 µL Proteinase K** stock solution, mix by vortexing, and incubate at **60–65 °C overnight**.

7 Centrifuge the samples for **5 min** at **12,000 x *g*** at **room temperature** in order to remove any unsoluble cell material. Proceed with the clear supernatant.

8 Sample A pellet: Add **300 µL Buffer B3** and **300 µL isopropanol** to the clear supernatant and apply the sample successively to the NucleoSpin® Tissue Column. Centrifuge **1 min** at **6,000 x *g*** (RT). If the sample is not drawing through the matrix completely, please repeat the centrifugation step.

Sample B supernatant: Add **400 µL of isopropanol** to the clear supernatant and apply the sample successively to the NucleoSpin® Tissue Column. Centrifuge **1 min** at **6,000 x *g*** (RT). If the sample is not drawing through the matrix completely, please repeat the centrifugation step.

9 Add **500 µL Buffer B5** (including ethanol) to the spin column and centrifuge **1 min** at **6,000 x *g*** (RT). Discard the flow-through. Repeat this washing step.

10 After the two washing steps with Buffer B5, discard the flow-through, place the NucleoSpin® Tissue Column in the centrifuge tube again, and centrifuge **2 min** at **6,000 x *g*** (RT) in order to remove Buffer B5 completely.

11 Place the NucleoSpin® Tissue Column in a clean 1.5 mL centrifuge tube and elute the DNA with **100–200 µL preheated Buffer BE (70° C)**. After **2 min** incubation, centrifuge for **1 min** at **6,000 x *g*** (RT).