Using NucleoSpin® Tissue for the isolation of genomic DNA from semen (Rev. 03)

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

The use of the buffer **GuEX** (200 mL) is required. The buffer GuEX is prepared from 2 mL of a sterile 5 M Guanidine hydrochloride solution (should not be autoclaved), 2,1 mL of a 1M Tris-Cl (pH 8) solution, 1,05 mL of a 2M NaCl solution, 4,2 mL of 0,5 M EDTA solution, 0.2 mL of a 1M NaOH solution. Add water to a volume of 200 mL. The pH should be between 8 - 8.5.

Prepare the **Buffers B3**, **B5**, and the **Proteinase K** according to the user manual. The **Elution Buffer BE** (5 mM Tris/HCl, pH 8.5) can be prewarmed (70° C) before elution).

Procedure

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

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

2. Centrifuge the mixture for 4 minutes at 12,000xg 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 (step 6).

4. Add **700 µL of GuEX** buffer to the pellet (**sample A Pellet**), centrifuge for 4 minutes at 12,000xg 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 of Proteinase K** stock solution, mix by vortexing, and incubate at 60 - 65°C overnight.

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

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

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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 minute at 6000xg (RT). If the sample is not drawn 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 minute at 6,000xg (RT). If the sample is not drawn through the matrix completely please repeat the centrifugation step.

9. Add **500 µL Buffer B5** (including ethanol) to the spin column and centrifuge 1 minute at 6,000xg (RT). Discard the flowthrough. Repeat this washing step.

10. After the two washing steps with **Buffer B5** discard the flow through, place the **NucleoSpin® Tissue** column again in the centrifuge tube and centrifuge 2 minutes at 6,000xg (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 two minutes incubation, centrifuge for 1 minute at 6,000xg (RT). For amplification reactions 20 µL of this sample can be used.

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