

NucleoSpin® DNA Forensic – Support Protocol - Semen

Protocol Details

Application	Genomic DNA from semen	
Kit	NucleoSpin® DNA Forensic	
REF	740840 (.10/ .50/ .250)	
Protocol name	Support Protocol - Semen	Rev01



Protocol Steps

Steps	Procedure
1. Differential lysis	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	Centrifuge the mixture for 4 min at 12,000 x g at room temperature. The pellet contains sperm cells (sample A pellet) wherea the free DNA (from epithelial cells and leukocytes, sample B supernatant) is in the supernatant.
3. Supernatant removal	The supernatant (sample B supernatant) is removed carefully, transferred to a fresh processed separately (see step 6).
4. Wash pellet	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. Resuspend pellet	The pellet (sample A pellet) is resuspended in a minimum of 300 µL Buffer T1
6. Lysis	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	Centrifuge the samples for 5 min at 12,000 x g at room temperature in order to remove any insoluble cell material. Proceed with the clear supernatant.
8. Bind DNA	Sample A pellet: Add 580 µL Buffer FOB to the clear supernatant and apply the sample successively to the NucleoSpin® DNA Forensic 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 580 µL Buffer FOB to the clear supernatant and apply the sample successively to the NucleoSpin® DNA Forensic 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. Proceed with step 4 of the user manual **Sample A pellet:** Proceed with step 4 (Wash with FOW1) of the user manual.

Sample B supernatant: Proceed with step 4 (Wash with FOW1) of the user manual.

Additional Equipment

Equipment	Content	REF
Buffer GuEX	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-HCl (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 1 M NaOH solution. Add water to a volume of 200 mL. The pH should be between 8-8.5	-
Buffer T1	50 mL	740940.25
	100 mL	740940.100
	1000 mL	740940.1000

Trademarks

NucleoSpin® (MACHEREY-NAGEL),

