Supplementary protocol

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NucleoSpin® DNA RapidLyse (Rev. 01, August 2017)

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). Safety data sheets (SDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

NucleoSpin® DNA RapidLyse – Supplementary lysis information:

1 Lyse sample

+ 150 µL RLY

+ 10 µL Liquid Proteinase K

Place the sample into a 2 mL tube.

Note: Do not use 1.5 mL conical tubes. The shape of the tube will impair thorough mixing. Use common 2 mL tubes which will facilitate proper sample and lysis buffer agitation.

Add 150 µL Buffer RLY.

Note: While mechanical homogenization of the sample is unnecessary in most cases, for some materials (e.g. fibrous tissue) a homogenization step in Buffer RLY prior to lysis may be beneficial for obtaining an optimal yield.

Add 10 µL Liquid Proteinase K.

Incubate at 56 °C on a heated shaking device (e.g. thermomixer) at maximum speed for a maximum time of 1 hour, or until the sample appears visually lysed (e.g. mostly cleared of particulates).

Note: If the sample is incubated in a heated water bath or heating block without agitation, vortex the sample frequently to ensure optimal lysis conditions.

Make sure that the tissue sample is submerged in the lysis buffer during incubation!

Centrifuge the tube at 11,000 x *g* for approx. 5 s (short spin), in order to clean the lid.

Note: If unlysed sample material remains after lysis, an additional centrifugation step is recommended to recover a cleared lysate. In this case, centrifuge 30 s at 14,000 x g.

Continue with **step 2** (Adjust DNA binding conditions) from the NucleoSpin® DNA RapidLyse protocol for fresh, frozen, and ethanol-preserved samples (section 5.1)

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Lysis time depends upon sample material and may vary from a couple of minutes to one hour.

Sample material	Lysis time (optimal)	DNA yield (typical)	Specification
Cells	15 min	5 µg	10^6 Hela cells
Bacteria (Gram-negative)	15 min	9–10 µg	30 mg *Pseudomonas fluorescens* (wet weight)
Bacteria (Gram-positive)	60 min	5 µg	30–40 mg *Corynebacterium glutamicum* (wet weight)
Blood	30 min	1 µg	200 µl EDTA whole blood
Organs (kidney)	60 min	30 µg	10 mg mouse kidney

Table 1 Optimal lysis time and typical yield for different samples types.
Genomic DNA was isolated with the NucleoSpin ® DNA RapidLyse kit from the following: 10^6 Hela cells; 30 mg Gram-negative bacteria *Pseudomonas fluorescens*; 30–40 mg Gram-positive bacteria *Corynebacterium glutamicum*, and 200 µl whole blood treated with EDTA. DNA was measured via OD after extraction according to the protocol for fresh, frozen and ethanol-preserved samples. *Note: For 200 µl blood samples 2 x binding buffer RLB was used.*