Supplementary protocol

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Support protocol for isolation of DNA from lipid tissues using the kit NucleoMag® DNA Bacteria (Rev. 02, April 2023)

Protocol at a glance using NucleoSpin® Bead Tube Type D

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

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.5 of the manual). It is recommended using a Square-well Block for separation (see section 2.5 of the manual). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

For hardware requirements, refer to section 2.4.1 and 2.4.2 of the manual

1 Prepare sample

Place the lipid tissue sample to a NucleoSpin® Bead Tube Type D.

Up to approximately 40 mg of wet weight lipid tissue sample can be processed. Remove excess liquid (e.g., water, ethanol) from the sample, with a filter paper.

Add **40 µL Buffer LBP** (not included) and 100 µL **Buffer IME** to the sample.

2 Lyse sample

Add 10 µL of Liquid Proteinase K, 2.5 µL Liquid RNase A and close the tube.

Agitate the NucleoSpin® Bead Tube Type D on the MN Bead Tube Holder in conjunction with Vortex-Genie®, swing mill or similar device for 0.5–20 min (e.g., 20 min for the MN Bead Tube Holder).

Note: Optimal agitation duration, speed / frequency depends on the device used. For the MN Bead Tube Holder it is approximately 20 min; in a mixer mill MM200, MM300, MM400 (Retsch®), e.g., 1 min at 10 Hertz followed by 10 s at 20 Hertz is suitable (see section 2.4.3 of the manual). On a swing mill, position of the tube in the mill can considerably influence the result. Please refer to the instruction manual of the device used. Respect warnings in section 1.2 and 2.4.3 of the manual if other devices are intended to be used!

Centrifuge the NucleoSpin® Bead Tube D for **30 s** at **11,000 x *g*** to clean the lid.

Attention: Do neither centrifuge at higher g-force, nor for longer than 30 s because this might damage the NucleoSpin® Bead Tubes.

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3 **Adjust binding conditions**

Add **600 µL Buffer LBP** (not included) and mix (e.g., vortex for 3 s).

Note: Steel beads should be agitated in the tube; some residual pellet (cell debris) may remain on the bottom of the tube.

Centrifuge for **30 s** at **11,000 x *g***.

Note: This centrifugation step is performed in order to clean the lid and sediment steel beads and cell debris.

Attention: Do neither centrifuge at higher g-force, nor for longer than 30 s because this might damage the NucleoSpin® Bead Tubes.

4 **Bind DNA to NucleoMag® B-Beads**

Transfer up to 600 µL lysate to a Square-well block. Avoid aspiration of cellular debris.

Note: Individual tubes strips can be removed from the Rack of Tube Strips allowing an easier access for supernatant aspiration

Add **24 µL of NucleoMag® B-Beads** and **320 µL of Binding Buffer IMB**. Mix by pipetting up and down 6 times and **shake** for **5 min at room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for **5 min** at **room temperature**.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Squarewell Block on the NucleoMag® SEP magnetic separator. Wait at least **5 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet might not be visible in this step. Remove supernatant from the opposite side of the well.

5 Proceed with step 5 of detailed protocol for isolation of DNA from microbes (see section 5.2 of the manual).