Supplementary protocol

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NucleoSpin® DNA Stool for Enrichment of host DNA from stool samples (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). Safety data sheets (SDS) can be downloaded from www.mn-net.com/MSDS.

Background

The vast majority of DNA isolated from feces origins from bacteria that colonize the stool material. Only a small portion of the DNA origins form the host itself. For some applications, the concentration of host DNA within the total DNA, using the standard protocol of the NucleoSpin® DNA Stool Kit as well as the NucleoSpin® 96 DNA Stool Kit may be too low and an enrichment of host DNA relative to bacterial DNA is desired.

The standard protocol of the NucleoSpin® DNA Stool Kit as well as the NucleoSpin® 96 DNA Stool Kit is intentional designed for harsh lysis conditions so that even robust gram positive bacterial get lysed. Especially the mechanical lysis step releases DNA from bacterial cells. In contrast, the release of the host DNA from the sample material requires much milder lysis conditions. In order to improve the ratio of host DNA relative to the bacterial DNA it is therefore necessary to reduce the stringency of the lysis step in the protocol.

NucleoSpin® DNA Stool Protocol for the enrichment of host DNA

1 Prepare sample

Transfer 180–220 mg of human stool material to an MN Bead Tube Type A.

Add 850 µl Buffer ST1, for the NucleoSpin® 96 DNA Stool Kit add 950 µl ST1

Close the MN Bead Tube and shake it horizontally for 2–3 seconds to mix stool sample and lysis buffer before putting it on a heat incubator.

Note: For animal stool samples different amounts of sample material and total lysis volume may be required.

2 Lyse sample

Incubate MN Bead Tube for 5 min at 70°C.

Agitate the MN Bead Tube in the MN Bead Tube Holder on a Vortex-genie®2. Pulse-vortex the samples 5 times for 1–2 sec. at maximum speed at room temperature.

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Proceed with the standard protocol in the NucleoSpin® DNA Stool Handbook, starting with step **3 Precipitate contaminants** without additional changes.

For the NucleoSpin® 96 DNA Stool protocol, centrifuge **3 mi**n at **13,000 x *g***, then proceed with step **3 Precipitate contaminants** without additional changes.