NucleoMag® DNA Microbiome

Magnetic bead based isolation of genomic DNA from microorganisms from soil, stool and biofilm samples on the MultiEX 032 workstation

Application benefits

Experience fast and flexible nucleic acid purification with the NucleoMag® DNA Microbiome kit and the MultiEX 032 system, featuring:

- Verified methods ensuring a reliable automated purification process
- Consistent unbiased extraction of DNA high in yield and purity
- Increasing throughput capability, processing up to 32 samples in parallel
- No programming or complex setup required: Verified and pre-installed methods available

Keywords

Soil, stool, feces, biofilms, metagenomics, bacteria, inhibitor removal, MN Bead tubes, microorganisms, metagenomics, genomic DNA, magnetic beads, magnetic beads, MultiEX 032

MultiEX 032

Scripts are pre-installed and biologically verified on the MultiEX 032 workstation.

Introduction

Microbes are the most ancient, abundant and diverse forms of life on earth. They colonize and proliferate every surface or organism – oftentimes in complex communities. Microorganisms define and modulate the health of their hosts and the entire ecosystem. One of the most important procedures in microbiome and metagenomics research is the extraction of high quality DNA representative of all species present in a sample. The MACHEREY-NAGEL NucleoMag® DNA Microbiome kit is designed to purify DNA from a variety of sample inputs, such as stool, soil and biofilms. Its patented inhibitor removal technology ensures efficient removal of contaminants from even challenging soil or fecal samples for superior downstream performance. In this Application Note, we demonstrate the advantages of automated DNA purification from microbial communities using the NucleoMag® DNA Microbiome on the MultiEX 032 platform. To ensure an efficient lysis, even from hard to lyse organisms, we combined the NucleoMag® DNA Microbiome with mechanical homogenization by bead beating to achieve an unbiased lysis.

NucleoMag® DNA Microbiome	
Technology	Magnetic beads
Sample material	Biofilm samples (incl. swabs), Soil, Stool
Target molecules	DNA
Elution volume	50 – 200 µL
Sample numbers on MultiEX 032	32 samples with 2 plates and 4 tip combs
Processing time	Approx. 50 min (excluding lysis)

MultiEX 032	
Technology	Automated magnetic rod system
Sample number	1 – 32 samples
Deck positions	Two plate positions with four rows of magnetic rods
Capacity	Volume per well: 50 µL to 1000 µL
Size / footprint	349 × 325 × 390 mm (width x depth x height)
Ease of use	10-inch color touch screen
Contamination control	Built-in UV lamp for disinfection

Material and Methods

Full disruption of sample material is required to acquire optimal yields of DNA from the samples. Therefore, 200 mg of various soil samples were transferred to MN Bead Tubes Type A and mixed with lysis buffer MI1 and RNase. The addition of Enhancer SX ensures optimal conditions to bind the DNA. Sample disruption was performed on MN Bead Tube Holder in combination with a Vortex-Genie® 2 for 4 – 5 minutes operating at full speed. After brief centrifugation 400 µL supernatant was transferred and contaminants were precipitated using Buffer MIc. Afterwards 500 µL of cleared lysates were transferred to column 1 of the extraction plate.

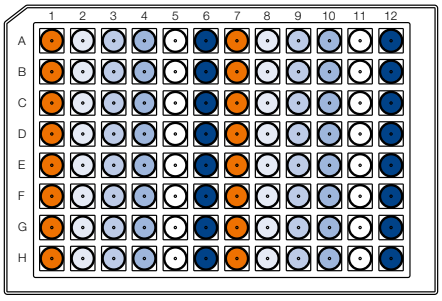
All further buffers were aliquoted in their respective wells as stated in the plate layout below. The plate was placed inside the MultiEX 032, the tip covers attached and the automation protocol started. Binding of nucleic acids was enabled by adjustment with Binding Buffer MI2. Subsequently the NucleoMag® B-Beads were washed to remove contaminants and salts using wash buffers MI3, MI4, and 70 % ethanol respectively. After air drying, highly pure DNA was eluted in 100 µL low-salt elution buffer MI5. All mixing and separation steps from binding to elution were carried out by the MultiEX 032 instrument.

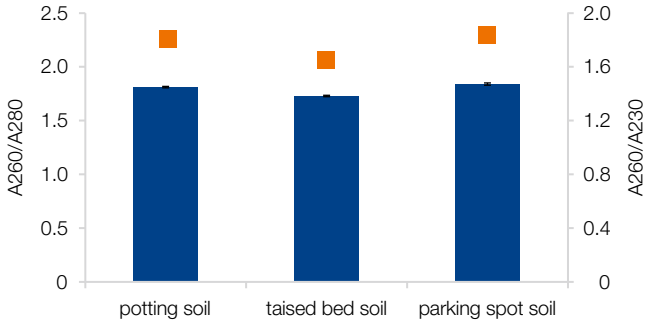
Plate layout

Column	Reagents
● 1 | 7	Lysate (500 µL), NucleoMag® B-Beads (25 µL), Binding Buffer MI2 (310 µL)
○ 2 | 8	Wash Buffer MI3 (600 µL)
● 3 | 9	Wash Buffer MI3 (600 µL)
● 4 | 10	Wash Buffer MI4 (600 µL)
○ 5 | 11	70 % Ethanol (600 µL)
● 6 | 12	Elution Buffer MI5 (100 µL)

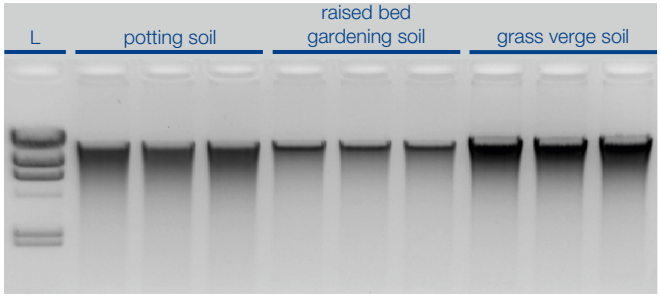
MN Bead Tubes Type A (DNA and nuclease free)

- 2 mL screw cap tubes
- Prefilled with 0.6 – 0.8 mm ceramic beads
- Compatible with common bead-beating devices



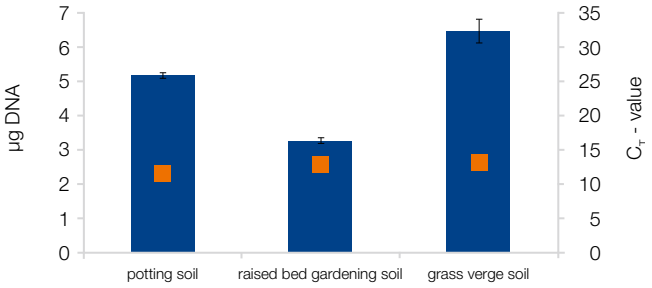


Application data



DNA extraction from different soil samples

DNA extraction was conducted from three different soil samples in biological triplicates of 200 mg using the NucleoMag® DNA Microbiome kit on MultiEX 032 system. High DNA quality and integrity was visualized via gel electrophoresis (1 % TAE-gel) shown on the picture above, revealing consistently high-molecular DNA. The gel bands show a low degree of fragmentation of genomic DNA due to careful but effective mechanical lysis via bead beating. The automated extraction on the MultiEX 032 allows for consistent DNA purification between replicates while remaining an easy to use workflow for the NucleoMag® DNA Microbiome kit.



qPCR and photometric measurment of DNA from different soil samples

The same samples shown on the gel were analyzed via photometrical measurement. The variation between replicates (n= 3) was very low, leading to near invisible error bars, because of standard deviations being under 0.01 for both ratios A260/A280 (blue bars) and A260/A230 (orange squares). The NucleoMag® DNA Microbiome kit was able to achieve excellent purities for all three challenging soil samples. The yield (blue bars) does vary depending on the soil type and therefore the abundance of microbial communities. Never the less the qPCR amplification of gram-positive and gram-negative bacteria using the BioLine SensiFast™ SYBR Lo-ROX kit on an Applied Biosystems® 7500 Real-Time PCR System show very low C_T-values, because of the bias-free isolation of all microorganisms present in the sample.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® DNA Microbiome	Magnetic bead based kit for the isolation of genomic DNA from microorganisms in soil, stool and biofilm (swab) samples	96 preps 384 preps	744330.1 744330.4
MN Bead Tubes Type A	2 mL tubes with 0.6 – 0.8 mm ceramic beads; for homogenization of soil, sediments, and stool (DNA and nuclease free)	50 pieces	740786.50
96 Deep-well plates	96-Deep-Well plates for processing of NucleoMag® kits with magnetic rod systems, e. g. MultiEX 032	25 pieces	744955
MultiEX 032	Magnetic rod system for bead based automated nucleic acid purification	1 – 32 samples	*

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* For more detailed information, please reach out to your local distributor.