

MACHEREY-NAGEL Application Note 06/2024 · Bioanalysis

NucleoSpin® 96 Soil

Automated isolation of total DNA from diverse soil types on the flowbot® ONE workstation

Application benefits

Experience enhanced nucleic acid purification workflows with the NucleoSpin® 96 Soil kit and flowbot® ONE, featuring:

- Verified methods ensuring a semi automated purification process.
- Consistent recovery of total DNA with reliable reproducibility in both yield and purity.
- High throughput capability, processing up to 96 samples in parallel.
- Accessible protocols through MACHEREY NAGEL's technical automation support at automation-bio@mn-net.com.

Keywords

Total DNA, Nucleic acid extraction, Automated DNA purification, inhibitor removal, soil, microbiome, omics, vacuum, Flowbot ONE, Flow Robotics automation system, laboratory protocol optimization

Flow Robotics flowbot® ONE

The flowbot® ONE Workstation was equipped with the NucleoVac 96 vacuum manifold an external vacuum pump and digital vacuum regulator

Introduction

Microorganisms, the most ancient, abundant, and diverse life forms on Earth, inhabit and proliferate across surfaces and organisms, often forming intricate communities. The objective of microbiome studies is to comprehensively understand their roles, functions, community structures, and interactions with their environments and hosts. A critical step in microbiome and metagenomic research involves extracting high-quality DNA that accurately represents all species present in a sample. The MACHEREY-NAGEL NucleoSpin® 96 Soil kit facilitates high-throughput, automation-friendly isolation of microbial DNA from various soil samples. Its buffer chemistry and Inhibitor Removal Plate ensure effective elimination of contaminants, even from challenging samples, resulting in superior downstream performance due to the efficient removal of inhibitors.

In this Application Note, we showcase the benefits of automated DNA purification from microbial communities using the NucleoSpin® 96 Soil kit on the flowbot® ONE platform. The procedure combines mechanical ceramic bead-based sample homogenization (MN Bead Tubes Type A or MN 96 Bead Plate Type A) with semi-automated DNA isolation, allowing for unbiased lysis and maximum DNA yields with convenience and reliability.

NucleoSpin® 96 Soil	
Technology	Silica membrane
Sample material	Soil, sediment and sludge samples
Target molecules	Total DNA
Fragment size	50 bp – approx. 50 kbp
Sample numbers on flowbot® ONE	96 samples with 1000 µL 8-channel pipette configuration

Flowbot® ONE	
Technology	Automated liquid handling platform equipped with electronic pipettes
Sample numbers	1-96 samples
Deck positions	Configurable platform with 12 deck slots + tip waste
Pipetting volume	2 pipette modules (choose between 1, 4 and 8-channel)
	Volume ranges: 1-20 μL, 2-200 μL and 10-1000 μL

Material and Methods

For a comprehensive and unbiased assessment of microbial content in samples, we recommend mechanically homogenizing the specimen, wherein 200-500 mg of various soil samples are transferred to MN Bead Tubes Type A (containing ceramic beads) and mixed with lysis buffer and optional RNase A. Soil samples benefit from the addition of Enhancer SX to optimize DNA release. Due to the high variety of soil composition the two lysis buffer systems offer a high degree of flexibility in order to choose the most efficient lysis buffer for your samples. Sample disruption occurs on the MN Bead Tube Holder, paired with a Vortex-Genie® 2 operating at full speed for 4-5 minutes. Subsequently, a precipitation step efficiently removes contaminants and inhibitors. All subsequent DNA purification steps (inhibitor removal filtration, binding, wash steps, drying, elution) are executed on the flowbot® ONE with an integrated NucleoVac 96 manifold. All pipetting steps were carried out by the flowbot® ONE, and vacuum pressure was controlled by the external digital regulator. Manual interventions were performed for reassembling the vacuum manifold and vacuum pump controle. The integration of fully automatic vacuum controle is currently under development at Flow Robotics.

Options for sample homogenization

MN Bead Tubes Type A

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



MN 96 Bead Plate Type A

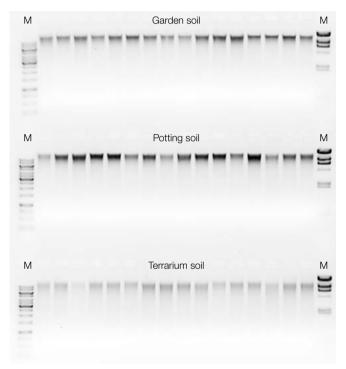
- Rack of prefilled tube strips (8 × 12)
- Prefilled with 0.6-0.8 mm ceramic beads
- Compatible with common plate disruption device



Single bead tubes or bead plates

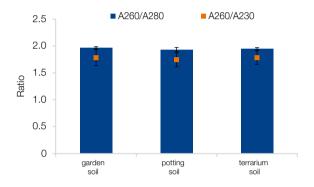
For optimal DNA yields, a complete disruption of sample material is necessary and can be performed with e.g., MN Bead Tubes Type A or MN 96 Bead Plates Type A. These accessories contain ceramic beads, resulting in efficient disruption of microbial cells in soil, stool and other materials used for microbiome analysis.

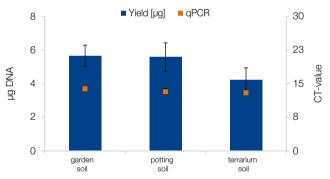
Application Data





DNA quality and integrity was visualized via gel electrophoresis (10 µL per lane, 1 % TAE-gel), revealing consistently high-molecular DNA with a low degree of fragmentation. High quality DNA was extracted from garden soil, potting soil and terrarium soil using the NucleoSpin® 96 Soil kit on the flowbot® ONE system. These results demonstrate the efficacy of the NucleoSpin® 96 Soil kit and the flowbot® ONE system in achieving reliable and high-quality DNA isolations from soil samples.





Purity and reliable qPCR performance of isolated DNA

DNA extraction was conducted from garden soil, potting soil and terrarium soil using the NucleoSpin® 96 Soil kit on the flowbot® ONE system. DNA purity and quantity was determined photometrically and subsequent qPCR analysis (depicted by orange squares), targeting the bacterial 16 s rRNA gene, was conducted using the SensiFast™ SYBR Lo-ROX kit from BioLine on an Applied Biosystems® 7500 Real-Time PCR System. Results show a consistent extraction and amplification of DNA even from challenging sample material, indicating the efficient removal of inhibitors and bias-free lysis of microorganisms.

Ordering information

Product	Specifications	Quantity	REF
NucleoSpin® 96 Soil	Isolation of total DNA from diverse soil types in proven 96-well plate format; including	2 × 96 preps	740787.2
	NucleoSpin® Inhibitor Removal Plate, NucleoSpin® Soil Binding Plate, buffers and plastic consumables (MN Wash Plate, MN square-well Block and Rack of tube Strips)	4 × 96 preps	740787.4
MN Bead Tube Type A	DNA- and nuclease-free 2 mL tubes with 0.6-0.8 mm ceramic beads; for homogenization of soil, sediments, and stool	1 × 50 pieces	740786.50
MN 96 Bead Plate Type A	Rack of prefilled tube strips (8 \times 12) containing 0.6 – 0.8 mm ceramic beads for homogenization	1 × 1 piece	740850.1
		1 × 4 pieces	740850.4
		1 × 24 pieces	740850.24
Flow Robotics flowbot® ONE	Automated platform for automated liquid handling and sample preparation	1-96 samples	Flow Robotics*

NucleoSpin® is a registered trademark of MACHEREY NAGEL (contact: automation-bio@mn-net.com); Flowbot® is a registered trademark of FlowRobotics; Applied Biosystems® is a registered trademark of

"For more detailed information, please visit www. flow-robotics.com. To contact FlowRobotics Sales or to schedule a demo, please email info@flow-robotics.com.