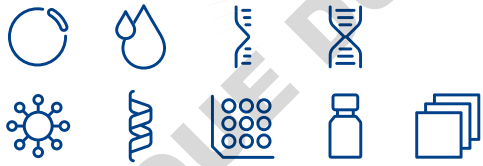
Bioanalysis

MACHEREY-NAGEL

User manual



DNA from soil, stool and biofilm samples

■ NucleoMag® DNA Microbiome

DOMINIQUE DUTSCHER SAS

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1 Components

1.1 Kit contents

	NucleoMag® DNA Microbiome	
REF	**1 × 96 preps 744330.1**	**4 × 96 preps 744330.4**
NucleoMag® B-Beads	2 × 1.5 mL	12 mL
Lysis Buffer MI1	125 mL	4 × 125 mL
Enhancer SX	2 × 10 mL	2 × 50 mL
Precipitation Buffer MIc	25 mL	2 × 50 mL
Binding Buffer MI2	40 mL	2 × 75 mL
Wash Buffer MI3	200 mL	3 × 200 mL
Wash Buffer MI4	125 mL	250 mL
Elution Buffer MI5	30 mL	125 mL
User manual	1	1

1.2 Reagents, consumables and equipment to be supplied by user

Reagents:

- 70 % ethanol (v/v) (absolute or non-denatured ethanol)

Consumables:

- Disposable pipet tips (aerosol barrier pipets tips are recommended to avoid cross-contaminations)

General:

- Personal protection equipment (e.g., lab coat, gloves, goggles)

Product	REF	Pack of
Magnet for magnetic beads separation		
e.g. NucleoMag® SEP (suitable for 96-(deep)-wel plates)	744900	1
e.g. NucleoMag® SEP Mini (suitable for 1.5 – 2 mL tubes)	744901	1
Separation plate for magnetic beads separation,	740481	4
e.g. Square-well Block (96-well block with 2.1 mL square-wells)	740481.24	24

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Product	REF	Pack of
Sample homogenization system		
MN Bead Tubes Type A e.g., Bead Tubes containing 0.6 – 0.8 mm ceramic beads; suitable in conjunction with MN Bead Tube Holder or mixer mills*	740786.50	50
MN Bead Tube Holder e.g., Tube Holder for Vortex-Genie® instrument and a 3-inch platform in order to house up to 12 bead tubes	740469	1
MN 96 Bead Plate Type A e.g., Rack of prefilled tube strips (12 strips with 8 tubes each) containing 0.6 – 0.8 mm ceramic bead; suitable in conjunction with mixer mills*	740850.1 740850.4 740850.24	1 4 24
High throughput consumables		
Round-well Block (low, U-bottom) e.g. Round-well Block with U-bottom for inhibitor precipitation.	740482 740482.20	4 20
Elution plate for collecting purified nucleic acids, e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24
For use of kit on KingFisher™ instruments: e.g., 96-well Accessory Kit B for KingFisher™ (Square-well Blocks, Deep-well tip combs, Plates for 4 × 96 NucleoMag® DNA/RNA Water preps using KingFisher® 96/Flex platform)	744951	1 set
For digestion of RNA Liquid RNase A	740397	2 × 1.25 mL

1.3 About this user manual

It is strongly recommended that first time users of the NucleoMag® DNA Microbiome kit read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used as a tool for quick referencing while performing the purification procedure.

All technical literature is available at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

*If using a bead mill, respect warnings, in the NucleoSpin® Bead Tubes user manual!

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1.4 Automation support

MN extraction kits are designed for streamlined automation, offering compatibility with a range of leading open robotic systems. Whether you're using magnetic rod systems or liquid handlers like Hamilton, Tecan, Eppendorf, or other platforms, our kits ensure efficient and reliable extraction processes. Reach out to us for comprehensive support and tailored automation solutions, making your extraction experience seamless and effortless.

Questions about MACHEREY-NAGEL's scripting support or automation service?

Please contact us for personal assistance:

Phone: +49 2421 969 333
Email: support@mn-net.com

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2 Product description

2.1 The basic principle

The **NucleoMag® DNA Microbiome** kit is designed for the isolation of DNA from soil, stool and biofilm samples. The procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by mechanical disruption using MN Bead Tubes Type A, MN 96 Bead Plates Types or other Bead Beating Tubes. Subsequently, contaminants are precipitated using Buffer MIc. For binding of nucleic acids to the paramagnetic beads, Binding Buffer MI2 and the NucleoMag® B-Beads are added to the transferred and cleared lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers MI3, MI4 and 70 % ethanol. Residual ethanol from previous wash steps is removed by air drying. Next, highly purified DNA is eluted with elution buffer MI5 and can be used directly for downstream applications. The **NucleoMag® DNA Microbiome** kit can be used either manually, or automated on standard liquid handling instruments and automated magnetic separators.

We can provide personalized support, protocol information, or verified scripts for numerous platforms. For more information, please contact our technical support or visit ***www.mn-net.com/automation***.

2.1.1 Kit specifications

Kit specifications at a glance	
Parameter	**NucleoMag® DNA Microbiome**
Technology	Magnetic bead technology
Format	Highly reactive superparamagnetic beads
Sample material	Soil, stool and biofilms (incl. swab samples)
Sample amount	50 – 200 mg
Typical yield	Varies by sample and disruption device
Elution volume	50 – 200 µL
Preparation time	40 – 120 min / 96 preps (excl. lysis)*
Processing	Manual or Automated
Use	For research use only

2.2 Sample materials

2.2.1 Stool samples

The NucleoMag® DNA Microbiome kit is optimized for processing 180 – 220 mg of stool samples. For stool samples from animals, it can be beneficial to reduce the amount of sample material to achieve optimal results.

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Very dry stool samples like rabbit or mouse feces may absorb lysis buffer, resulting in an insufficient sample volume after the first centrifugation step. In these cases it is recommended to reduce the amount of stool material (e.g., to 60 – 80 mg) and increase the total lysis volume to 1 mL. A 1:1 mixture of Buffer MI1 and nuclease free water is recommended for these stool samples (see also section 2.4 for detailed information about input material and lysis conditions). For difficult stool samples like lipid, polysaccharide, or protein rich stool, a reduction of starting material might also improve the lysis efficiency and the purity of the DNA. It is recommended in such cases to start the extraction with 60 – 80 mg sample material.

Human stool samples may also contain undigested food matter (e.g., crop or fruit husks, undigested seeds). These particles should not be transferred to the MN Bead Tubes.

The chemical lysis by Buffer MI1 can be supported by an optional heat incubation step at 70 °C for 5 min within the MN Bead Tube before mechanical disruption, as well as by an RNase A treatment during lysis. This step improves lysis and solubilization of stool compounds. It is necessary to shake each sample horizontally for 2 – 3 seconds after addition of Buffer MI1 before placing it in the heat incubator in order to mix the stool material and the buffer.

2.2.2 Soil samples

The NucleoMag® DNA Microbiome kit is optimized for processing up to 200 mg of soil samples. In some cases higher sample amounts are also feasible. However, for some soil samples, lowering the sample amount may lead to better results.

Due to the highly varying composition of different soil samples (e.g. organic/inorganic matter, humic substances, metal ions, polysaccharides, pH, etc.), the optional use of the Enhancer SX during mechanical disruption may lead to better results. The use of the Enhancer SX is more suitable for samples consisting predominantly of minerals.

Very dry material can soak up large volumes of lysis buffer. In this case, either reduce the amount of sample material or add additional lysis buffer for the mechanical disruption. If possible remove foreign material like leaves, stones, or twigs (e.g., by sieving) as well as excess of water (e.g., by discarding the supernatant after spinning down sediment samples).

2.2.3 Biofilm samples

The NucleoMag® DNA Microbiome kit is designed for processing up to 200 mg of biofilm samples, including microbial mats or swab samples.

Biofilm samples can be either extracted directly (e.g. scrapings, cutting) or can be extracted from cotton or synthetic swabs. It is recommended, to increase the amount of lysis buffer in order to fully submerge the swab head during mechanical disruption.

The chemical lysis by Buffer MI1 can be supported by an optional heat incubation step at 70 °C for 5 min with the MN Bead Tubes before mechanical disruption. This step improves lysis and solubilization of stool compounds. It is necessary to shake each sample horizontally for 2 – 3 seconds after addition of Buffer MI1 before placing it in the heat incubator in order to mix the stool material and the buffer.

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2.3 Lysis and disruption of sample material

In order to obtain optimal yields of DNA from sample material, a complete disruption of the sample material is necessary. Sample disruption efficiency depends on the following parameters and can be achieved by following the suggestions outlined in the subsequent sections, as well as in section 2.2.

2.3.1 Disruption device

The following devices are compatible with MN Bead Tubes or MN 96 Bead Plates. Please check whether the available tube adapters can accommodate MN Bead Tubes or MN 96 Bead Plates prior to starting the procedure. MN Bead Tube Holder (for MN Bead Tubes) in combination with the Vortex-Genie® 2 (recommended). Mixer Mill MM200, MM300, MM400 (Retsch®) (suitable for MN Bead Tubes or MN 96 Bead Plates; Please check the compability of the available adaptors prior to starting the procedure). If other disruption devices (section 1.2) are intended to be used, consider section 2.2.3 and read carefully the WARNING note.

2.3.2 Type of bead tubes or bead plates

Bead type, disruption time, and frequency/speed must be optimized for a give sample to obtain maximal DNA yield and quality.

Type	Recommended for	REF
MN Bead Tubes Type A (0.6 – 0.8 mm ceramic beads)	Soil, sediment, biofilm and stool samples	740786.50
MN 96 Bead Plate Type A (0.6 – 0.8 mm ceramic beads)	Soil, sediment and stool samples	740850.1 740850.4 740850.24

Do not moisten or contaminate the inner rim of the individual tubes of the MN 96 Bead Plate during sample filling. It is recommended to use disposable weighing funnels (e.g. Sigma-Aldrich Weighing funnel, size XS, Z509329) in order to minimize the risk of cross-contaminations during sample filling.

2.3.3 Time and frequency of disruption

The following recommendations have been established for the MN Bead Tube Holder in combination with a Vortex-Genie® 2 or a Retsch® Mixer Mill MM300 operating at highest frequency (30 Hertz). **For using other disruption devices, and other sample materials, time and frequency have to be optimized.**

Time and frequency of disruption using MN Bead Holder on a Vortex-Genie® 2

Sample material	MN Bead Tube	Disruption time / speed
Soil	MN Bead Tubes Type A	Approx. 4 min, full speed
Stool	MN Bead Tubes Type A	Approx. 5 – 10 min, full speed
Biofilm	MN Bead Tubes Type A	Approx. 5 – 10 min, full speed

Time and frequency of disruption using a Retsch Mixel Mill MM300

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Sample material	MN Bead Tube	Disruption time / speed
Soil	MN Bead Tubes Type A	Approx. 1 – 2 min, 30 Hz
Stool	MN Bead Tubes Type A	Approx. 1 – 2 min, 30 Hz
Biofilm	MN Bead Tubes Type A	Approx. 1 – 2 min, 30 Hz
Soil	MN 96 Bead Plate Type A	Approx. 2 × 3 min *, 30 Hz
Stool	MN 96 Bead Plate Type A	Approx. 2 × 3 min *, 30 Hz
Biofilm	MN 96 Bead Plate Type A	Approx. 2 × 3 min *, 30 Hz

Note: Performance and stability testing has been conducted on the MN Bead Tubes Type A, B, C, D and on the MN 96 Bead Plate Type B and D on a Retsch® Mixer Mill MM300 at highest frequency (30 Hertz) for up to 15 minutes (MN Bead Tube Type A, B and C; MN 96 Bead Plate Type B and D) or up to 30 min (MN Bead Tube Type D). For optimal sample disruption, avoidance of DNA fragmentation, and highest DNA yield, see recommendation table above for adequate disruption conditions. Other disruption devices (see e.g. section 2.3.1) will require different settings regarding frequency and duration for optimal performance with the selected sample material. Please note that the position of the tube within the machine (Retsch® Mixer Mill) is important for optimal performance! Please consult instruction manual of the respective device.

WARNING: Many modern disruption devices can cause very high energy input on the MN bead tubes or on the MN 96 Bead Plate. Depending on bead tube or bead plate type and content (beads, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause damage to the individual tubes or plates! Never use the MN 96 Bead Plate with less than 12 tube strips. It is the responsibility of the user to perform initial stability test for the used bead tubes or plates under the conditions used!

Perform initial test with water instead of lysis buffer and moderate machine setting (low frequency, short time) in order to avoid spillage of sample and lysis buffer in case of tube breakage. Integrity and tightness of the tube need to be controlled after every run.

2.4 Handling of magnetic beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. During automation, a premix step before aspirating the beads buffer mixture from the tube is recommended to keep the beads resuspended.

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* Re-orient MN 96 Bead Plates vertically for 180° after the disruption time. Ensure that all tubes are sealed properly before and after each disruption. Samples which have been the closest to the machine body should now the furthest apart.

Do not contaminate or moisten the inner rim of the individual tubes during sample filling.

2.5 Automation – Liquid handling systems

The NucleoMag® DNA Microbiome can be automated on various liquid handling platforms using the NucleoMag® SEP (MN REF: 744900) in combination with the Square-well Block (MN REF: 740481) and with a suitable shaking device for optimal resuspension during binding, washing and elution steps. Additionally, a gripper tool is required for fully automated use on liquid handling workstations. The gripper needs to transfer the plate to the magnetic separator for the separation of the beads and then to the shaker module for resuspension of the beads. The complete resuspension of the magnetic beads during the extraction is essential for a reliable performance and should be checked during the validation on liquid handling systems. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times.

2.5.1 Adjusting the shaker settings

Efficient resuspension of the NucleoMag® B-Beads is essential for reliable extraction and is dependent on the specifications of the used shaking device (e.g. speed and orbit). When using a plate shaker for the binding, washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and specific shaking devices to prevent cross-contamination from well to well. It is recommended to loosen the magnetic bead pellet on a shaking device before dispensing buffer easing the resuspension of magnetic beads. Proceed as follows

Adjusting shaker speed for binding and wash steps:

- Load 1000 µL dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing step.

Adjusting shaker speed for the elution step:

- Load 100 – 200 µL dyed water to the wells of the collection plate and proceed as described above.

2.6 Automation – Automated magnetic separators

The NucleoMag® DNA Microbiome can be automated on different automated magnetic separation platforms using the specific consumables of the respective instrument. The magnetic beads are usually resuspended by the movement of the plastic sheath (tip-comb) covering the magnetic rods. Following the binding, washing and elution steps the beads are collected again with the magnetic rods. Buffers and individually components have to be dispensed in most of the cases individually. Please make sure, not to exceed the maximum filling volume per reaction container according to the manufacturers specifications.

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2.7 Elution procedures

Purified DNA can be eluted directly with the supplied Elution buffer MI5 (5 mM Tris/HCl, pH 8.5). Elution can be carried out in a volume of ≥ 50 µL. It is essential to cover the NucleoMag® B-Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, higher elution volumes might be necessary to cover the whole pellet.

The eluted DNA is ready to use for all standard downstream applications. In most cases the concentrated DNA can be used as PCR-template without further dilution for highest sensitity.

2.8 Evaluation of DNA yield and quality

The most common method to determine the DNA yield is UV-VIS spectroscopy. The DNA concentration in the final eluate can be calculated from its absorption maximum at 260 nm (A_{260}). However, this calculation assumes the absence of any other compound that absorbs UV light at 260 nm. Some contaminations significantly contribute to the total absorption at 260 nm and can therefore lead to an overestimation of the actual DNA concentration.

Purity ratio A_{260}/A_{280}

The main indicator of DNA purity is the ratio A_{260}/A_{280}, which should be between 1.7 and 1.9. Values below 1.7 indicate protein contamination.

Purity ratio A_{260}/A_{230}

Another indicator of DNA purity is the ratio of the absorption at 260 nm and 230 nm. A_{260}/A_{230} should be higher than 2.0 for pure DNA and can be accepted down to about 1.5. Ratios around or even below 1.0 indicate impurities in the DNA eluate, which could be of different nature as several compounds absorb at these wavelengths.

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3 Storage conditions and preparation of working solutions

Attention: *Buffer MI4 contains chaotropic salt! Wear protective clothing, gloves and goggles!*

- Store Buffer MI2 upon arrival at 4 °C.
- All other components of the **NucleoMag® DNA Microbiome** kit should be stored at room temperature (15 – 25 °C) and are stable until: see package label.

All buffers are delivered ready to use.

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4 Safety instructions

When working with the **NucleoMag® DNA Microbiome** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***http://www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in buffer MI4 can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoMag® DNA Microbiome** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

WARNING: *The use of other disruption devices like FastPrep® System (MPBiomedicals), Precellys® (Bertin Technologies), MagNA™ Lyser (Roche), TissueLyser (QIAGEN), Bullet Blender® (Next Advance), Mini-Beadbeater™ (Biospec Products), Speed Mill (Analytik Jena), or similar devices might cause bead tube or bead plate destruction. Such disruption devices can cause high mechanical stress on the bead tubes or bead plates. Depending on bead tube / bead plate type and content (beads like steel balls, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause destruction of the bead tubes or bead plates. If using such a disruption device, it is the responsibility of the user to perform initial stability tests to ensure stability of bead tubes or bead plates during the individual experimental setup (e.g., intensity of agitation). See also section 2.3.*

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

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5 Protocol overview

The NucleoMag® DNA Microbiome kit offers a high flexibility allowing the isolation of DNA from soil, stool and biofilm samples. The following overview depicts the main differences between the sample pretreatment procedures.

Soil samples	Stool samples	Biofilm samples
Transfer sample to MN Bead Tube or MN Bead Plate		
Add lysis buffer (optional Enhancer)	Add lysis buffer (optional RNase A / optional heat incubation)	
Mechanical disruption		
Precipitate debris and contaminants		
Bind nucleic acid to NucleoMag® B-Beads		
1 st Washing step		
2nd Washing step		
3rd Washing step		
4th Washing step		
Air dry		
Elution		

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6 Protocol for isolation of DNA from soil, stool and biofilm samples using MN Bead Tubes

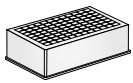
6.1 Protocol at a glance

Carefully read the detailed protocol (section 6.2) as well as section 2 before starting the procedure. This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.5 – 2.6). It is recommended to use a Square-well Block for separation (see section 1.2). 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 automation platforms.

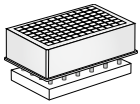
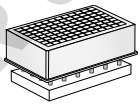
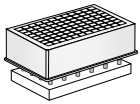
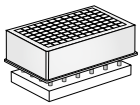
For hardware requirements and additional consumables, refer to sections 2.3 and 1.2.

Before starting the preparation:

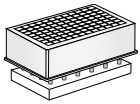
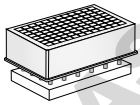
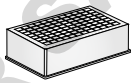
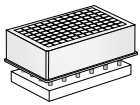
- Check section 2.2 and 2.3 for additional information about the amount of starting material and sample material preparation.
- Check if 70 % ethanol was prepared according to section 1.2.

Prepare sample	**MN Bead Tube Type A** **Up to 200 mg sample material** **700 µL MI1** *Optional (Soil): 150 µL Enhancer SX* *Optional: 2.5 µL Liquid RNase A* *Optional (Stool / Biofilm): 70 °C, 5 min* **Agitate on a swing mill or similar device for 1 – 10 min**	
Precipitate debris	**Incubate for 5 min at RT** **11,000 x *g*, 5 min** **Transfer supernatant to a fresh tube**	
Precipitate contaminants	**150 µL MIc** **Vortex 5 s** **10 min, 2 – 8 °C** **11,000 x *g*, 5 min** **Transfer supernatant into a fresh tube**	
Bind DNA to NucleoMag® B-Beads	**Up to 500 µL lysate** **25 µL NucleoMag® B-Beads** **310 µL MI2**	
	Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	

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	Remove supernatant after 5 min separation	
Wash with MI3	**Remove Square-well Block from NucleoMag® SEP** **600 µL MI3**	
	Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
	Remove supernatant after 2 min separation	
Wash with MI3	**Remove Square-well Block from NucleoMag® SEP** **600 µL MI3**	
	Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
	Remove supernatant after 2 min separation	
Wash with MI4	**Remove Square-well Block on NucleoMag® SEP** **600 µL MI4**	
	Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
	Remove supernatant after 2 min separation	
Wash with 70 % EtOH	**Remove Square-well Block on NucleoMag® SEP** **600 µL 70 % EtOH**	
	Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	

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	Remove supernatant after 2 min separation	
Dry the beads	**10 – 15 min at RT**	
Elute DNA	**Remove Square-well Block from NucleoMag® SEP** **50-100 µL MI5**	
	Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
	Separate 2 min and transfer DNA into elution plate	

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6.2 Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.5 – 2.6). It is recommended to use a Square-well Block for separation (see section 1.2). 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 automation platforms.

For hardware requirements and additional consumables, refer to sections 2.3 and 1.2.

Before starting the preparation:

- Check section 2.2 and 2.3 for additional information about the amount of starting material and sample material preparation.
- Check if 70 % ethanol was prepared according to section 1.2.

1 Prepare sample

Transfer up to **200 mg sample material** to a **MN Bead Tube Type A.**

Add **700 µL Lysis Buffer MI1**.

Note: Increase the total lysis buffer volume up to 1 mL in case of very dry sample material as the sample will soak up a partial volume of the buffer.

Optional (soil samples): Add 150 µL Enhancer SX

Optional: Add 2.5 µL of Liquid RNase A (not provided)

Optional (stool and biofilm samples): Close the Tube, agitate horizontally for 2 – 3 seconds and incubate for 5 min at 70 °C.

Note: See section 2.2 for more for more information about the amount of starting amterial and the recommended lysis procedure.

2 Lyse sample

Agitate the **MN Bead Tube Type A** on the **MN Bead Tube Holder** in conjunction with Vortex-Genie®, swing mill or similar device for 1 – 10 min (e.g. 4 min for the MN Bead Tube Holder).

Note: Optimal agitation duration, speed / frequency depends on the device used. See section 2.3 for more information about recommended lysis and homogenization conditions for different sample materials. 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 the warning in section 2.3 if other devices are intended to be used!

Incubate the tubes for **5 min at RT**, allowing fine debris to settle.

Centrifuge for **5 min** at **11,000 x *g***.

Note: Prolong the centrifugation for additional 5 min, in case of excessive foam formation.

Attention: Do neither centrifuge at higher g-force, nor for longer than 10 min because this might damage the MN Bead Tube.

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Transfer the **supernatant** carefully to a fresh 2 mL microcentrifuge tube with lid (not provided).

Note: Transfer as much lysate as possible to the 2 mL microcentrifuge tube. Avoid transferring material from the pellet or material, which floats on top of the lysate. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.

3 Precipitate contaminants

Add 150 µL of Buffer MIc, close the lid and vortex for 5 s.

Incubate for 10 min at 2 – 8 °C (preferably on ice).

Centrifuge for 5 min at 11,000 x *g*.

4 Bind DNA to NucleoMag® B-Beads

Transfer **up to 500 µL lysate to a Square-well block**.

Note: Avoid transferring material from the pellet or material.

Add **25 µL of NucleoMag® B-Beads** and **310 µL of Binding Buffer MI2**. 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.

Pipette gently in order to avoid foaming. If using electronic pipettes, use only 40 % of the total volume as mixing volume.

Note: Be sure to resuspend the NucleoMag® B-Beads before usage. Vortex storage bottle until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well 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 Wash with MI3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer MI3** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1 – 3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 – 5 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

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6 Wash with MI3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer MI3** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1 – 3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

7 Wash with MI4

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL MI4** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1 – 3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

8 Wash with 70 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL 70 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1 – 3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

9 Air dry magnetic beads

Air dry the magnetic beads for **10 – 15 min** at room temperature.

10 Elute DNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add desired volume of **Elution buffer MI5 (50 – 100 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5 min** at room temperature. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5 – 10 min** at **room temperature**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified DNA to suitable elution plate.

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7 Support Protocol for MN 96 Bead Plate Type A

7.1 Detailed protocol

This protocol is designed for the use of the MN 96 Bead Plate Type A during mechanical sample disruption, as an alternative to the MN Bead Tube. Please note, that the use of the MN 96 Bead Plate is only recommended for sample materials, which can be easily portioned and handled.

- For hardware requirements and additional consumables, refer to sections 2.3 and 1.2.

1 Prepare sample

Transfer up to **200 mg sample material** to each individual tube of the MN 96 Bead Plate Type A.

Note: Do not moisten or contaminate the inner rim of the individual tubes of the MN 96 Bead Plate during sample filling. It is not recommended to the MN 96 Bead Plate for adhesive or gluey samples. It is recommended to use disposable weighing funnels (e.g. Sigma-Aldrich Weighing funnel, size XS, Z509329) in order to minimize the risk of cross-contaminations during sample filling.

Add **700 µL Lysis Buffer MI1** to each sample and seal the tubes strips tightly with the cap strips.

Note: Increase the total lysis buffer volume up to 900 µL, in case of very dry sample material, as the sample will soak up a partial volume of the buffer.

Optional (soil samples): Add 150 µL Enhancer SX

Optional: Add 2.5 µL of Liquid RNase A (not provided)

Optional (stool and biofilm samples): Close the Tube, agitate horizontally for 2 – 3 seconds and incubate for 5 min at 70 °C. Ensure that each well is sealed properly before, during and after heat incubation.

Note: See section 2.2 for more for more information about the amount of starting material and the recommended lysis procedure.

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2 Lyse sample

Agitate the MN 96 Bead Plate Type A on a swing mill or similar device for 2 × 0.5 – 5 min at 30 Hertz.

Note: Re-orient MN 96 Bead Plates vertically for 180° after the first disruption time. Samples which have been the closest to the machine body should be now the furthest apart. Ensure that each well is sealed properly before and after each disruption.

Note: Optimal agitation duration, speed / frequency depends on the device used. See section 2.3 for more information about recommended lysis and homogenization conditions for different sample materials. 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 the warning in section 2.3 if other devices are intended to be used!

Incubate the tubes for **5 min at RT**, allowing fine debris to settle.

Centrifuge for **5 min** at **4,000 x g**.

Attention: Do neither centrifuge at higher g-force, nor for longer than 5 min because this might damage the MN 96 Bead Plate.

Transfer the **supernatant** carefully to a fresh 96-(deep)-well block (e.g. Round-well Block low (U-bottom) or Square-well Block.

Note: Transfer as much lysate as possible to the fresh 96-well plate. Avoid transferring material from the pellet or material, which floats on top of the lysate. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.

3 Precipitate contaminants

Add 150 µL of Buffer MIc to each sample, mix by pipetting up and down 3 times or **shake** for **5 min** at **room temperature**.

Incubate for 10 min at 2 – 8 °C (preferably on ice).

Centrifuge for 5 min at 4,000 x *g*.

4 Bind DNA to NucleoMag® B-Beads

Transfer **up to 500 µL lysate to a Square-well block** and proceed with step 4 of detailed protocol in section 6.2

Note: Avoid transferring material from the pellet or material.

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8 Support Protocol for samples stored in DNA/ RNA Shield™

8.1 Detailed protocol

This protocol is designed for the use of the NucleoMag® DNA Microbiome with samples stored in the storage solution DNA/RNA Shield™.

- For hardware requirements and additional consumables, refer to sections 2.3 and 1.2.

1 Prepare sample

Prepare the sample according to the DNA/RNA Shield™ sample preparation guidelines e.g., by mechanical lysis using the MN Bead Tubes Type A or the MN Bead Plate Type A.

Incubate the tubes for **5 min at RT**, allowing fine debris to settle.

Centrifuge for **5 min** at **11,000 x *g***.

Attention: Do neither centrifuge at higher g-force, nor for longer than 10 min because this might damage the MN Bead Tube.

2 Transfer 200 – 400 µL supernatant carefully to a fresh reaction tube or suitable vessel.

Note: Transfer as much lysate as possible to the 2 mL microcentrifuge tube. Avoid transferring material from the pellet or material, which floats on top of the lysate. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.

Precipitate contaminants

Add 150 µL of Buffer MIc to each sample, close the lid and vortex for 5 s. Alternatively, mix by pipetting up and down 3 times or **shake** for **5 min** at **room temperature**.

Incubate for 10 min at 2 – 8 °C (preferably on ice).

Centrifuge for 5 min at 11,000 x *g*.

Proceed with step 4 of the standard protocol (binding step)

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9 Appendix

9.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA/RNA yield	*Insufficient mechanical treatment of filter membrane* • For most sample sources, we recommend mechanical disruption with ceramic beads for 4 – 5 min on the MN Bead Tube Holder or with commercial bead mills, mixers or homogenizers. See sections 2.3 for further recommendations. *Insufficient extraction of nucleic acids during lysis* • To obtain higher yields of nucleic acids, an additional heat incubation for 5 min at 70 °C can be performed prior mechanical treatment. *Sample contains too much RNA* • Add 10 µL RNase A solution (12 mg/mL) to the lysis buffer following mechanical lysis. If this is not successful, add the enzyme to the cleared lysate and incubate for 30 min at 37 °C. *Insufficient Elution buffer volume* • Bead pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step* • Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of following wash steps and elution step. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation was too short or aspiration speed was too high.

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Problem	Possible cause and suggestions
Low purity / Low sensitivity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down. Increase the wash buffer volumes from 600 µL to 900 µL in each step.
Suboptimal performance of DNA in downstream applications	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down. • Repeat washing step with 70 % ethanol. *Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Ethanol evaporation from wash buffers* • Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs. *Insufficient removal of inhibitor/brownish eluates* • Reduce the amount of sample material input.
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

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Problem	Possible cause and suggestions
Cross contamination	*Contamination of the rims* • Do not moisten the rims of the Square-well Block when transferring the lysate. If the rim of the wells is contaminated, seal the Square-well Block with Self adhering PE Foil (see ordering information, section 9.2) before starting the shaker.

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9.2 Ordering information

Product	REF	Pack of
NucleoMag® DNA Microbiome	744330.1 744330.4	1 × 96 preps 4 × 96 preps
NucleoSpin® Bead Tube Type A	740786.50	50
MN Bead Tube Holder	740469	1
MN 96 Bead Plate Type A	740850.1 740850.4 740850.24	1 4 24
NucleoMag® SEP	744900	1
NucleoMag® SEP Mini	744901	1
Square-well Blocks	740481 740481.24	4 24
Round-well Block (low, U-bottom)	740482 740482.20	4 20
Self adhering PE Foil	740676	50 sheets
Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24
96-well Accessory Kit B for KingFisher™ (set consists of Square-well Blocks, Deep-well tip combs, Elution Plates; for 4 × 96 NucleoMag® DNA Microbiome preps using KingFisher™ 96/Flex platform)	744951	1 set
For digestion of RNA: Liquid RNase A	740397	2 × 1.25 mL
96 Deep-well plates for magnetic rod systems	744955	25
8-well Tip Combs for magnetic rod system	744960	50
8-well Accessory Kit magnetic rod systems	744961	1 Set

Visit ***www.mn-net.com*** for more detailed product information.

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9.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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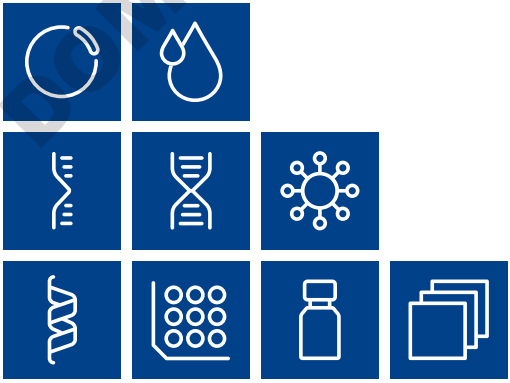
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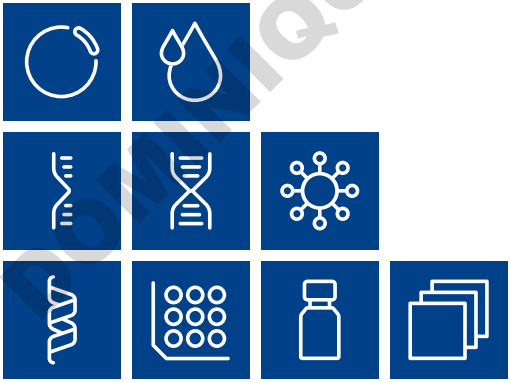
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