Bioanalysis

MACHEREY-NAGEL

User manual

Genomic DNA from soil

■ NucleoSpin® Soil

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MACHEREY-NAGEL

www.mn-net.com

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Genomic DNA from soil

Protocol at a glance (Rev. 12)

NucleoSpin® Soil

1	**Prepare sample**		MN Bead Tube Type A 250 – 500 mg sample material 700 µL SL1 **or** SL2
2	**Adjust lysis conditions**		150 µL Enhancer SX
3	**Sample lysis**		Horizontally vortex 5 min at RT or use other homogenizers according to manufacturers protocol
4	**Precipitate contaminants**		11,000 x *g*, 2 min 150 µL SL3 Vortex 5 s 0 – 4 °C, 5 min 11,000 x *g*, 1 min
5	**Filter lysate**		Load supernatant on NucleoSpin® Inhibitor Removal Column (red ring) 11,000 x *g*, 1 min
6	**Adjust binding conditions**		250 µL SB Vortex 5 s
7	**Bind DNA**		Load 550 µL sample on NucleoSpin® Soil Column (green ring) 11,000 x *g*, 1 min Load remaining sample 11,000 x *g*, 1 min
8	**Wash silica membrane**		**1st** 500 µL SB 11,000 x *g*, 30 s **2nd** 550 µL SW1 11,000 x *g*, 30 s **3rd** 650 µL SW2 Vortex 2 s 11,000 x *g*, 30 s **4th** 650 µL SW2 Vortex 2 s 11,000 x *g*, 30 s
9	**Dry silica membrane**		11,000 x *g*, 2 min
10	**Elute DNA**		30 – 100 µL SE RT, 1 min 11,000 x *g*, 30 s

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1 Components

1.1 Kit contents

	NucleoSpin® Soil		
REF	**10 preps 740780.10**	**50 preps 740780.50**	**250 preps 740780.250**
Lysis Buffer SL1	30 mL	60 mL	250 mL
Lysis Buffer SL2	30 mL	60 mL	250 mL
Lysis Buffer SL3	10 mL	10 mL	50 mL
Enhancer SX	3 mL	10 mL	50 mL
Binding Buffer SB	10 mL	60 mL	250 mL
Wash Buffer SW1	6 mL	30 mL	150 mL
Wash Buffer SW2 (Concentrate)*	6 mL	25 mL	100 mL
Elution Buffer SE**	13 mL	13 mL	60 mL
MN Bead Tubes Type A	10	50	250
NucleoSpin® Inhibitor Removal Columns (red rings)	10	50	250
NucleoSpin® Soil Columns (green rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
Collection Tubes (2 mL, lid)	10	50	250
User manual	1	1	1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer SE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- Isopropanol (when working with samples which are stabilized in Zymo DNA/RNA Shield)
- 96 – 100 % ethanol

Consumables

- 1.5 mL microcentrifuge tubes
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.6)
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended that first time users of the **NucleoSpin® Soil** 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 only as a supplemental tool for quick referencing while performing the purification procedure.

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

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

2 Product description

2.1 The basic principle

The sample material is resuspended in Lysis Buffer SL1 or SL2, supplemented with the Enhancer SX, and mechanically disrupted using ceramic beads.

Proteins and PCR inhibitors are precipitated with Lysis Buffer SL3 and subsequently pelleted by centrifugation together with the ceramic beads and undissolved sample material. The supernatant is taken off and cleared by passing it through a NucleoSpin® Inhibitor Removal Column.

DNA binding conditions are then adjusted by addition of Binding Buffer SB to the flow through and the lysate is loaded onto a NucleoSpin® Soil Column.

Residual humic substances, especially humic acids, and other PCR inhibitors are removed by efficient washing with Binding Buffer SB and Wash Buffers SW1/SW2. After a drying step, ready to use DNA can be eluted with Elution Buffer SE (5 mM Tris/HCl, pH 8.5).

2.2 Kit specifications

- The **NucleoSpin® Soil** kit is designed for the isolation of high molecular weight genomic DNA from microorganisms like Gram positive and Gram negative bacteria, archaea, fungi, and algae in soil, sludge, and sediment samples.
- Suitable for soils from forest, bog, farmland, grassland, etc.
- Suitable for samples stabilized in Zymo DNA/RNA Shield (isopropanol required)
- Suitable for stool samples.
- The kit offers two special lysis buffers, **Buffer SL1** and **Buffer SL2**, which can be combined with the chemical additive **Enhancer SX** to guarantee highest possible yields with excellent purity for all types of sample material.
- Efficient mechanical lysis of the sample material is achieved by bead beating using the ceramic **MN Bead Tubes Type A**.
- The optimized buffer chemistry and the **NucleoSpin® Inhibitor Removal Column** completely remove humic substances and other PCR inhibitors typically present in soil and sediment samples.
- 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 sensitivity.

Table 1: Kit specifications at a glance	
Parameter	**NucleoSpin® Soil**
Format	Mini spin columns
Sample material	< 500 mg soil or sediment
Typical yield	2 – 10 μg
Elution volume	30 – 100 μL
Preparation time	90 min/10 preps
Binding capacity	50 μg
Use	For research use only

2.3 Relevance of humic substances as PCR inhibitors

Humic substances are produced by bacteria, fungi, and protozoa in soil, sediments and waters during the degradation of plant or other organic matter. They consist of very high molecular weight compounds with undefined structures. Building blocks are mainly heterocyclic aromatic compounds that are linked by ether or ethoxy groups and which carry hydroxyl-, methoxy-, carbonyl-, or carboxyl groups.

According to their solubility in water they are divided into humin, humic acids, and fulvic acids. The completely insoluble and black humin has an average molecular weight of around 300,000 g/mol. The dark brown to grey colored humic acids are slightly smaller. They carry a lot of hydroxyl and carboxyl groups and are therefore mainly soluble at neutral or alkaline pH. The only slightly yellow to light-brown colored fulvic acids with an average molecular weight of 2,000 g/mol are soluble under alkaline as well as under acidic conditions.

Due to the high molecular weight and the mainly polyanionic nature of humic substances most purification methods do not distinguish between these molecules and DNA. For the same reason they act as extremely potent PCR inhibitors. Even smallest amounts of humic substances can inhibit for example DNA polymerases or restriction enzymes and result in a complete failure of enzymatic downstream applications.

Frequently, the problem is circumvented by dilution of the isolated DNA prior to PCR analysis. However, this results in a significantly reduced sensitivity because low abundance DNA may be lost completely.

Thus, highest DNA yields with as little PCR inhibitor contaminations as possible are of utmost importance for any DNA analysis of soil samples.

2.4 Amount of starting material

NucleoSpin® Soil is suitable for processing 250 – 500 mg of sample material. However, do not fill the MN Bead Tube Type A higher than the 1 mL mark (including the ceramic beads) to ensure sufficient head space for an efficient mechanical disruption.

Usually a reduction of starting material also helps to improve the lysis efficiency and to increase the purity of the DNA.

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 up to the 1.5 mL mark of the MN Bead Tube Type A.

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).

2.5 Choice of lysis buffer

Due to the highly varying composition of different soils (organic matter, inorganic matter, humic substances, metal ions, polysaccharides, pH, etc.), it is impossible to obtain best results in DNA yield and purity for all sample types with only one single lysis buffer system.

There are several parameters that can be adjusted in a way that lysis works perfect for one sample but fails with another. Therefore, the NucleoSpin® Soil kit is equipped with two lysis buffers SL1 and SL2 and an Enhancer SX.

Those three components allow a perfect fine tuning for every type of soil sample for maximum yield and purity. Unfortunately, for the reasons given above there is no way to predict the best choice of lysis buffer for a specific sample. This can only be determined experimentally. Therefore, **both lysis buffers should be tested in parallel** for each new sample material. Usually Buffer SL1 in combination with Enhancer SX is more suitable for soil consisting predominantly of minerals while Buffer SL2 is more suitable for soil with a high amount of organic carbon.

After mixing the sample with lysis buffer in the MN Bead Tube Type A, the Enhancer SX is added routinely to the sample prior to the mechanical homogenization. This buffer ensures the highest possible DNA yield with most sample materials. However, in case of a very high humic acid content in the sample material, the Enhancer SX might also reduce the purity of the DNA by facilitating the release of humic acids into the lysate. Therefore, the volume of added Enhancer SX can be lowered from 150 μL to for example 10 μL or the buffer can be entirely omitted. This usually increases the purity (A_{260}/A_{230}) of the sample significantly (Table 2), might, however, lower the DNA yield (Figure 1).

Ideally, for a new sample material both lysis buffers **Buffer SL1 and SL2** should be tested **with and without adding Enhancer SX.** These initial four preparations will help you to find the ideal lysis condition for your special soil composition.

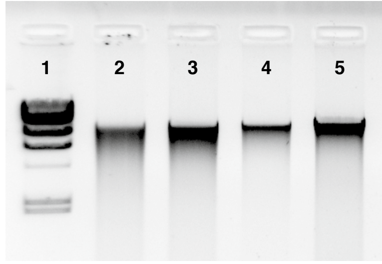


Figure 1: **Total DNA purified from wheat field soil with four different lysis buffer combinations**

20 of 100 µL eluate were analyzed on a 1 % TAE agarose gel:

Lane 1: Marker λ / HindIII

Lane 2: Lysis Buffer SL1

Lane 3: Lysis Buffer SL1 +Enhancer SX

Lane 4: Lysis Buffer SL2

Lane 5: Lysis Buffer SL2 +Enhancer SX

Table 1: Yields and purity ratios of DNA purified from wheat field soil

Buffer	**SL1**		**SL2**	
Enhancer SX	-	+	-	+
Yield	2.3 µg	2.3 µg	1.4 µg	3.1 µg
A_{260}/A_{280}	1.69	1.60	1.76	1.72
A_{260}/A_{230}	1.85	0.96	1.78	0.99

2.6 Mechanical sample lysis

A thorough mechanical lysis step is essential to break up the soil crumbs, to free the cells within the soil, and to break up cells and spores. Ceramic beads have proven to be most effective in combination with a bead mill, a FastPrep®-24 instrument (MP Biomedicals, set instrument to 5 m/s for 30 s), or an MN Bead Tube Holder, see ordering information. In most cases, however, this kind of equipment is not necessary. The same result can be achieved by taping the lysis tubes **horizontally** to a standard vortexer.

The lysis time should be as short as necessary to avoid shearing of DNA and to minimize the release of humic acids. Depending on the sample, however, it might be advantageous to increase the lysis time to 10, 20, or 30 min.

Homogenization and cell disruption should be performed at room temperature (18 – 25 °C) to avoid SDS precipitation in the lysis buffers. Overheating the sample, for example by prolonged bead beating in a bead mill or the FastPrep®-24 instrument, should be avoided to minimize liberation of humic acids.

2.7 Repeated extraction

For sample materials containing a high amount of microorganisms a single extraction step might not be sufficient to disrupt every cell and to release all DNA. Extracting the sample twice may help to increase DNA yield significantly.

Therefore, follow the protocol until the first centrifugation in step 4. But instead of adding SL3 directly to the MN Bead Tube Type A, transfer the supernatant to a new collection tube (not provided) and complete step 4 with this supernatant. Then repeat steps 1 – 4 with the same soil sample in the MN Bead Tube Type A. Filter both final supernatants of step 4 through a NucleoSpin® Inhibitor Removal Column as described in step 5. Add Binding Buffer SB to both filtrates according to step 6 and finally load both samples on one NucleoSpin® Soil Column according to step 7 in multiple loading steps.

Note that the supplied buffer volumes are calculated for only one extraction. The excess of Enhancer SX and Binding Buffer SB might not be sufficient to allow two extraction steps for all 10, 50, or 250 preps of the kit.

For more information, please visit our website:
https://www.mn-net.com/de/microbiome-information

2.8 Elution procedures

It is possible to adapt the elution method, temperature, and volume of elution buffer used for the subsequent application of interest. In addition to the standard method where an increase of DNA concentration can be achieved by reducing the elution volume from 100 to 30 μL, there are two options to increase the DNA yield:

- Heat the elution buffer to 80 °C.
- Perform two subsequent elution steps with fresh elution buffer.

2.9 How to interpret DNA yield and purity from UV-VIS

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}) based on the fact that an absorption of A_{260} = 1 corresponds to 50 µg/mL double stranded DNA. However, this calculation assumes the absence of any other compound that absorbs UV light at 260 nm. Any contamination with, for example, RNA, protein, or especially humic substances significantly contributes to the total absorption at 260 nm and therefore leads to an overestimation of the real DNA concentration.

Figure 2 shows a typical UV absorbance spectrum of pure DNA (solid line) exhibiting a peak at 260 nm, a decrease of absorption with a minimum at 230 nm, and only a moderate increase in absorption below 230 nm. In comparison, the spectrum of a sample that is contaminated with humic acids demonstrates only a small shoulder at 260 nm, it lacks the minimum at 230 nm, and the absorption sores up below 230 nm. In this case only a small part of the absorbance at 260 nm is caused by DNA, most of it is just the tailing absorption of the humic acid contamination. However, the calculated DNA yield seems to be higher in the contaminated sample. Thus, DNA yield determined by UV-VIS, might be distorted by co-purifying contaminants and we recommend to check the DNA yield also by agarose gel electrophoresis.

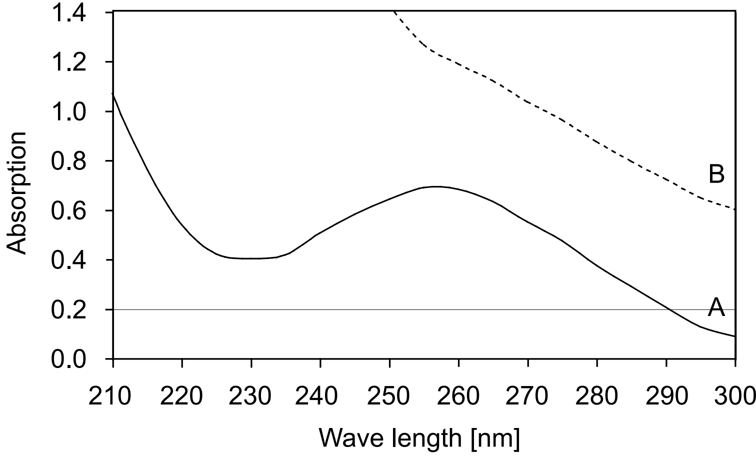


Figure 2:**UV-VIS quantification of A) pure DNA and B) contaminated DNA**

A) 7.7 µg in 100 µL, 1.84 A_{260}/A_{280}, 1.71 A_{260}/A_{230}

B) 9.3 µg in 100 µL, 1.35 A_{260}/A_{280}, 0.27 A_{260}/A_{230}

Purity ratio A_{260}/A_{230}

To facilitate the decision whether the yield as determined from A_{260} readings can be trusted or not, the ratio of the absorption at 260 nm and 230 nm can be used. The ratio A_{260}/A_{230} should be higher than 2.0 for pure DNA and is acceptable down to ratios of about 1.5. Smaller values around or even below 1.0, as shown in Figure 2, indicate significant amounts of impurities and the real DNA concentration is far below its calculated value.

Additionally, not only humic acids, but also proteins, saccharides, and other contaminants can be detected by a low A_{260}/A_{230} ratio.

Purity ratio A_{260}/A_{280}

Another indicator of DNA purity is the ratio A_{260}/A_{280}, which should be between 1.8 and 1.9. Values below 1.8 indicate protein contamination, whereas higher values indicate RNA contamination. However, this ratio should be treated with caution, since contamination with protein and RNA at the same time can compensate each other and result in a perfect A_{260}/A_{280}.

Agarose gel electrophoresis

As a consequence, the DNA should always be run on an agarose gel to verify the UV-VIS quantification especially if A_{260}/A_{230} and A_{260}/A_{280} are beyond the acceptable range. Figure 3 demonstrates that the contaminated sample B) of Figure 2 actually contains much less DNA than the pure sample A) in contrast to the UV-VIS results, which can easily be misinterpreted.

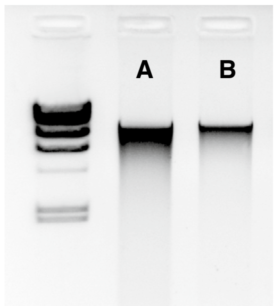


Figure 3:Gel analysis of A) pure and B) contaminated genomic DNA from soil

10 µL of each sample were run on a 1 % TAE agarose gel (1 h, 100 V). The larger gel band of pure DNA A) proves a higher yield and concentration compared to the contaminated DNA sample which is in contrast to the UV-VIS quantification (A: 7.7 µg/100 µL, B: 9.3 µg/100 µL).

2.10 Isolation of DNA from water samples

Additionally required equipment

- MN Bead Tubes 5 mL Type A for 45 – 50 mm filters (see ordering information)
- MN Bead Tube Holder 5 mL (see ordering information)
- 15 mL centrifuge tubes (for use with 45 – 50 mm filters)

Volume of water sample

The maximum volume of water sample that can be processed varies depending on the sample (e.g. source and quality) and filter membrane (e.g., type, diameter, pore size).

The turbidity of water samples can vary from clear to highly turbid, due to different concentrations of particulates. In general, high volumes of clear or potable drinking water can be processed, as there is a lower chance of filter clogging. A volume of 100 mL up to several liters might be processed.

Turbid water samples containing high levels of sediments or suspended particles, such as clay, silt, or other inorganic or organic matter, may lead to filter clogging. The use of a 0.45 μm filter is recommended for these samples types. Furthermore, it is advantageous to let samples sediment over time. In case a sedimentation is not desired or does not occur, a pre-filter on top of the membrane filter is recommended. MACHEREY-NAGEL offers a broad range of filter paper suitable for pre-filtration of large debris.

Suitable pre-filter	Properties	Particle retention	Diameter	REF
MN 619 G	slow filtration	approx. 2 – 7 μm	55 mm	440005
MN 616 G	medium filtration	approx. 4 – 12 μm	55 mm	483005
MN 617 G	fast filtration	approx. 7 – 12 μm	55 mm	494005

Storage of water sample

The most convenient way is the storage of filtered water samples. Therefore, filter the water sample through a membrane filter and store the filter directly within the appropriate amount of lysis buffer in the respective bead tube. Keep in mind that sodium dodecylsulfate in Buffer SL1 and SL2 will precipitate at lower temperatures. Incubate tubes at 37 °C until the SDS precipitates have redissolved before starting the preparation.

Filter Membranes

NucleoSpin® Soil is suitable for the nucleic acid extraction from both 25 mm and 47 mm filter membranes of different composition or origin. Disposable filter funnel units as well as reusable filter funnels are compatible and can be used with different filter membrane types. MACHEREY-NAGEL offers a broad range of different membrane filter types and sizes.

Type	Diameter	Pore size	Product	REF
Cellulose acetate (CA)	25 mm	0.2 µm	PORAFIL® CA	68000020025
		0.45 µm	PORAFIL® CA	68000045025
	47 mm	0.2 µm	PORAFIL® CA	68000020047
		0.45 µm	PORAFIL® CA	68000045047
Cellulose mixed esters (CM)	25 mm	0.45 µm	PORAFIL® CM	65100045025
	47 mm (sterile)	0.45 µm	PORAFIL® CM	65300045047
Cellulose nitrate (NC)	25 mm	0.2 µm	PORAFIL® NC	6570020025
		0.45 µm	PORAFIL® NC	6570045025
	47 mm	0.2 µm	PORAFIL® NC	6570020047
		0.45 µm	PORAFIL® NC	6570045047
Polycarbonate (PC)	25 mm	0.40 µm	PORAFIL® PC	676040025
Polyethersulfone (PES)	-	-		-

The choice of a suitable bead tube type highly depends on the filter membrane size.

- 25 mm filter membranes: MN Bead Tube Type A (0.6 – 0.8 mm ceramic beads, supplied)
- 47 mm filter membranes: MN Bead Tube 5 mL Type A (0.6 – 0.8 mm ceramic beads, see ordering information)

2.11 Isolation of DNA from samples stabilized in Zymo DNA/RNA Shield

The NucleoSpin® Soil kit can be combined with Zymo DNA/RNA Shield solution. Add Zymo DNA/RNA Shield to the soil sample in Bead Tube Type A until the sample is barely but completely submerged. Use as little Zymo DNA/RNA Shield solution as possible to keep the total volume low. 500 mg of soil sample usually require the addition of 500 μL Zymo DNA/RNA Shield.

Follow the protocol to step 6. Adjust binding conditions by addition of 250 μL Buffer SB according to step 6 and determine the total sample volume. Add 0.2 sample volumes of isopropanol to the sample and vortex for 5 s. For example: if the total sample volume after addition of Buffer SB is 1000 μL, add 200 μL isopropanol. Continue with step 7.

3 Storage conditions and preparation of working solutions

Attention:

Buffers SB and SW1 contain guanidinium thiocyanate and guanidine hydrochloride, respectively. Wear gloves and goggles!

Storage conditions:

- All kit components should be stored at 15 – 25 °C and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts. If precipitation occurs, incubate the bottle for several minutes at about 30 – 40 °C and mix well until the precipitate is dissolved.

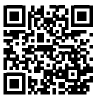
Before starting the first **NucleoSpin® Soil** procedure prepare the following:

- **Wash Buffer SW2:** Add the indicated volume of ethanol (96 – 100 %) to Buffer SW2 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Buffer SW2 is stable at 15 – 25 °C for at least one year.
- Sodium dodecyl sulfate (SDS) in Buffer SL1/SL2 may precipitate if stored at temperatures below 20 °C. If a precipitate is observed in Buffer SL1/SL2, incubate the bottle at 30 – 40 °C for several minutes and mix well

NucleoSpin® Soil			
REF	**10 preps 740780.10**	**50 preps 740780.50**	**250 preps 740780.250**
Wash Buffer SW2 (Concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol	100 mL Add 400 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® Soil** 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 ***www.mn-net.com/msds***).



Caution: Guanidin thiocyanate in Buffer SB and buffer SW1 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 **NucleoSpin® Soil** 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 but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

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.

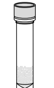
5 Protocol – purification of DNA from soil and sediment

Before starting the preparation:

- Check Lysis Buffer SL1 or SL2 for precipitated SDS. Dissolve any precipitate by incubating the buffer at 30 – 40 °C for 10 min and shaking the bottle every 2 min.

1 Prepare sample

See section 2.4 *and* 2.5 *for more information on the amount of starting material and the choice of lysis buffer. See section* 2.7 *for the repeated extraction of a sample to improve DNA yield.*



250 – 500 mg sample +700 µL SL1 or SL2

Transfer **250 – 500 mg** fresh **sample material** to a **MN Bead Tube Type A** containing the ceramic beads.

Important: **Do not fill** the tube **higher** than the **1 mL mark.**

Add **700 µL Buffer SL1** or **Buffer SL2.**

Note for very dry material: If the sample material soaks up too much lysis buffer, fill the MN Bead Tube Type A up to the 1.5 mL mark with fresh lysis buffer.

Note for very wet material: Remove excess liquid before addition of lysis buffer, if necessary after spinning down the sample.

2 Adjust lysis conditions

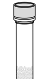
Add **150 µL Enhancer SX** and close the cap.

+150 µL SX

Note: Enhancer SX ensures the highest possible DNA yield. It can, however, also promote the release of humic acids. See section 2.5 on how to lower the volume or omit the buffer entirely in order to increase DNA purity.

3 Sample lysis

See section 2.6 *for more information on homogenization methods (e.g., FastPrep®-24 instrument, Vortex adapter).*



Vortex RT, 5 min

Attach the MN Bead Tubes **horizontally** to a vortexer, for example, by taping or using a special adapter.

Vortex the samples at **full speed** and **room temperature** (18 – 25 °C) for **5 min**.

4 Precipitate contaminants

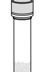
Centrifuge for **2 min** at **11,000 x *g*** to eliminate the foam caused by the detergent.

Note: The clear supernatant can be transferred to a new collection tube (not provided) prior to the following precipitation. This might result in more consistent yields from prep to prep and is highly recommended for carbonate containing samples. See also section 2.7 for repeated extraction of a sample to improve DNA yield.

Add **150 µL Buffer SL3** and vortex for **5 s**.

Incubate for **5 min** at **0 – 4 °C**.

Centrifuge for **1 min** at **11,000 x *g***.



11,000 x *g*, 2 min

+150 µL SL3

Vortex 5 s

0 – 4 °C, 5 min

11,000 x *g*, 1 min

5 Filter lysate

Place a **NucleoSpin® Inhibitor Removal Column** (red ring) in a Collection Tube (2 mL, lid).

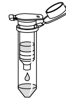
Load up to **700 µL** clear supernatant of step 4 onto the filter.

Centrifuge for **1 min** at **11,000 x *g***.

Note: With very wet samples (e.g., sediments) the volume of clear supernatant of step 4 can exceed 700 µL significantly. In this case transfer the NucleoSpin® Inhibitor Removal Column to a new collection tube (not provided) and load the remaining supernatant. Centrifuge for 1 min at 11,000 x g. Combine the flow throughs.

Discard the NucleoSpin® Inhibitor Removal Column.

If a pellet is visible in the flow through, transfer the clear supernatant to a new collection tube (not provided).



Load supernatant

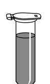
11,000 x *g*, 1 min

6 Adjust binding conditions

Add **250 µL Buffer SB** and close the lid.

Vortex for **5 s**.

Note: If samples were stabilized in Zymo DNA/RNA Shield, quantify total sample volume after addition of Buffer SB and add 0.2 volumes of isopropanol.



+250 µL SB

Vortex 5 s

7 Bind DNA

Place a **NucleoSpin® Soil Column** (green ring) in a Collection Tube (2 mL).

Load **550 μL sample** onto the column.

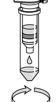
Centrifuge for **1 min** at **11,000 x *g***.

Discard flow through and place the column back into the collection tube.

Load the **remaining sample** onto the column.

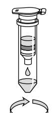
Centrifuge for **1 min** at **11,000 x *g***.

Discard flow through and place the column back into the collection tube.



Load 550 μL sample

11,000 x *g*, 1 min

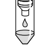


Load remaining sample

11,000 x *g*, 1 min

8 Wash and dry silica membrane

1 st wash



+500 µL SB

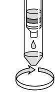
Add **500 µL Buffer SB** to the NucleoSpin® Soil Column.

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

Discard flow through and place the column back into the collection tube.

11,000 x *g*, 30 s

2nd wash



+550 µL SW1

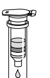
Add **550 µL Buffer SW1** to the NucleoSpin® Soil Column.

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

Discard flow through and place the column back into the collection tube.

11,000 x *g*, 30 s

3rd wash

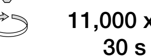


+650 µL SW2

Vortex 2 s

Add **650 µL Buffer SW2** to the NucleoSpin® Soil Column.

Close the lid and vortex for **2 s**. Centrifuge for **30 s** at **11,000 x *g***. Discard flow through and place the column back into the collection tube.

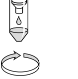


11,000 x *g*, 30 s

4th wash

+650 µL SW2

Vortex 2 s



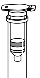
Add **650 µL Buffer SW2** to the NucleoSpin® Soil Column.

Close the lid and vortex for **2 s**. Centrifuge for **30 s** at **11,000 x *g***. Discard flow through and place the column back into the collection tube.

11,000 x *g*, 30 s

<u>Note:</u> The same collection tube is used throughout the entire washing procedure to reduce plastic waste. If new collection tubes are to be used for each step, see section 6.2 for ordering information.

9 Dry silica membrane



Centrifuge for **2 min** at **11,000 x *g***.

If for any reason, the liquid in the collection tube has touched the NucleoSpin® Soil Column after the drying step, discard flow through and centrifuge again.

11,000 x *g*, 2 min

10 Elute DNA

Place the NucleoSpin® Soil Column into a new microcentrifuge tube (not provided).

Add **30 μL** (for high concentration), **50 μL** (for medium concentration and yield), or **100 μL** (for high yield) **Buffer SE** to the column.

Do not close the lid and incubate for 1 min at room temperature (18 – 25 °C). Close the lid and centrifuge for **30 s** at **11,000 x *g***.

Note: Quantify DNA not only by UV-VIS but also run an agarose gel to verify yield and DNA quality (see section 2.9 for more information).

30 – 100 μL SE

RT, 1 min

11,000 x *g*, 30 s

5.1 Protocol – purification of DNA from water

Protocol for isolation of DNA from 25 mm Filters

Filter water sample through an appropriate 25 mm filter membrane (not provided, see ordering information) using a suitable filtration device.

Remove the filter membrane from the filtration device by using sterile forceps, roll the membrane into a cylinder (top side facing inwards) and insert into a MN Bead Tube Type A. Add 700 μL Buffer SL1 or SL2 and optionally up to 150 μL Buffer SX. Addition of Buffer SX is not recommended for water samples with a high concentration of humic acids.

Continue with step 3 of the protocol. Remove and discard filter using a sterile pipette tip.

Protocol for isolation of DNA from 47 mm Filters

Filter water sample through an appropriate 47 mm filter membrane (not provided, see ordering information) using a suitable filtration device.

Remove the filter membrane from the filtration device by using sterile forceps, roll the membrane into a cylinder (top side facing inwards) and insert it into a MN Bead Tube 5 mL Type A (see ordering information). Add 1400 μL Buffer SL1 or SL2 and optionally up to 300 μL Buffer SX. Addition of Buffer SX is not recommended for water samples with a high concentration of humic acids.

Vortex the MN Bead Tube 5 mL Type A on the MN Bead Tube Holder 5 mL (see ordering information) horizontally in conjunction with a Vortex-Genie® for 15 min at maximum speed.

Centrifuge the Bead Tubes at > 1000 x *g* for 2 minutes. Remove and discard filter using a sterile pipette tip.

Add 300 μL Buffer SL3 and vortex for 5 s. Incubate at 0 – 4 °C for 5 min.

Centrifuge the Bead Tubes at > 1000 x *g* for 5 minutes at 4 °C.

Load up to 650 µL supernatant into a NucleoSpin® Inhibitor Removal Column (red ring) and centrifuge at 11,000 x *g* for 1 min. Collect flow-through in a clean 15 mL centrifuge tube (not supplied). Repeat this step until the remaining supernatant is filtered using fresh NucleoSpin® Inhibitor Removal Columns (not supplied, see ordering information) for each repetition.

Add 500 µL Buffer SB to the pooled flow-through and vortex for 5 s. See ordering information for additional Buffer SB if needed.

Load up to 550 µL sample into a NucleoSpin® Soil Column (green ring) and centrifuge at 11,000 x *g* for 1 min. Discard flow-through. Repeat this step to load the remaining sample.

Continue with step 8 of the protocol.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor or no DNA yield	*Suboptimal lysis conditions* • Too much sample material was filled into the MN Bead Tube Type A. Too little head space does not allow the necessary motion of the beads to disrupt the sample. Use less sample material (see section 2.4 for more information). • Compare the yields obtained with Lysis Buffer SL1 and SL2 in parallel purifications each with and without addition of Enhancer SX to find the optimal lysis buffer conditions (see section 2.5 for more information). *Insufficient disruption and/or homogenization of starting material* • Shaking of the MN Bead Tube Type A was too weak or not long enough. Increase shaking time and velocity or use another shaking device (see section 2.6 for more information). Make sure that the MN Bead Tube Type A is fixed horizontally on the vortexer. *Reagents not applied or restored properly* • Always dispense exactly the buffer volumes given in the protocol! • Always follow closely the given instructions with regard to order and mode of mixing (shaking, vortexing, etc). • Add the indicated volume of ethanol (96 – 100 %) to Wash Buffer SW2 Concentrate and mix thoroughly (see section 3 for more information). • Store kit components at room temperature (18 – 25 °C). Storage at lower temperatures may cause salt precipitation. Check Lysis Buffer SL1 and SL2 for white precipitate. If precipitation occurred, incubate the bottle for 10 min at 30 – 40 °C and shake every 2 minutes until all precipitate is dissolved (see section 3 for more information). • Keep bottles tightly closed in order to prevent evaporation or contamination. *Sample material not stored properly* • Whenever possible, use fresh material.

Problem	Possible cause and suggestions
DNA is degraded	*Too harsh mechanical sample disruption* • Reduce intensity or incubation time of mechanical sample lysis. *DNA is degraded by DNases* • Add at least 10 – 15 μL Enhancer SX to the lysate.
Suboptimal performance of DNA in downstream experiments	*DNA yield was overestimated* • If DNA eluates are not completely free of contaminants (e.g., RNA, protein, humic substances) UV-VIS quantification based on A_{260} is not reliable due to the contribution of the contaminants to the absorption at 260 nm *Carry-over of ethanol or salt* • Make sure to dry the silica membrane and the NucleoSpin® Soil Column completely before elution to avoid carry-over of ethanolic Wash Buffer SW2. • Check if Buffer SW2 has been equilibrated to room temperature (18 – 25 °C) before use. Washing at lower temperatures decreases the efficiency of salt removal. *Contamination with PCR inhibitors* • The DNA purity can be increased by lowering the amount of starting material (see section 2.4 for more information). • Enhancer SX can facilitate the release of humic substances. Reduce Enhancer SX to 10 μL or omit the buffer entirely (see section 2.5 for more information). • Make sure to carefully follow the washing instructions. • Dilute DNA 1:10 to reduce concentration of inhibitors.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Soil	740780.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® DNA Stool	740472.10 / .50	10 / 50 preps
NucleoSpin® Microbial DNA	740235.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® DNA Lipid Tissue	740471.10 / .50	10 / 50 preps
NucleoSpin® DNA Insect	740470.10 / .50	10 / 50 preps
Buffer SB	740785.50	60 mL
Buffer SL1	740781.30	30 mL
Buffer SL2	740782.30	30 mL
Buffer SL3	740783.50	50 mL
Enhancer SX	740784.50	50 mL
MN Bead Tube Holder	740469	1 piece
MN Bead Tubes Type A (0.6 – 0.8 mm ceramic beads, recommended for soil and sediments)	740786.50	50 pieces
MN Bead Tubes Type B (40 – 400 μm glass beads, recommended for bacteria)	740812.50	50 pieces
MN Bead Tubes Type C (1 – 3 mm corundum, recommended for yeast)	740813.50	50 pieces
MN Bead Tubes Type D (3 mm steel beads, recommended for insects)	740814.50	50 pieces
MN Bead Tubes Type E (40 – 400 μm glass beads and 3 mm steel beads, recommended for hard-to-lyse bacteria within insect samples)	740815.50	50 pieces
MN Bead Tubes Type F (1 – 3 mm corundum and 3 mm steel beads, recommended for challenging tissues, e.g., spleen, or lung tissue)	740816.50	50 pieces
MN Bead Tubes Type G (5 mm steel beads, recommended for plant material)	740817.50	50 pieces

Product	REF	Pack of
Collection Tubes (2 mL)	740600	1000
MN Bead Tubes 5 mL Type A e.g., 5 mL Bead Tubes containing 0.6 – 0.8 mm ceramic beads; suitable in conjunction with MN Bead Tube Holder 5 mL or mixer mill*	740799.50	50
MN Bead Tube Holder 5 mL e.g., 5 mL Tube Holder for Vortex-Genie® instrument and a 3-inch platform in order to house up to 5 bead tubes	740459	1
NucleoSpin® Inhibitor Removal Columns	740789.50 / .250	50 / 250

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

6.3 Product use restriction / warranty

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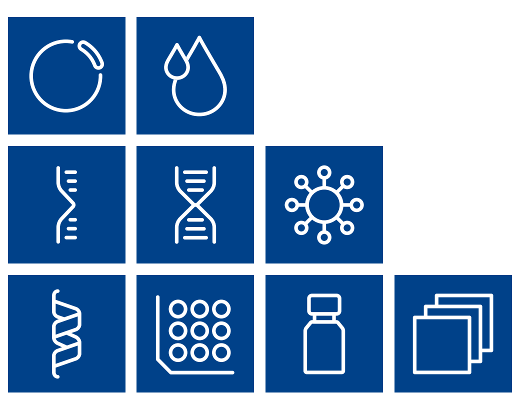

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