MACHEREY-NAGEL

User manual

Bioanalysis

Genomic DNA from soil

- NucleoSpin® 96 Soil

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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoSpin® 96 Soil	
REF	**2 × 96 preps 740787.2**	**4 × 96 preps 740787.4**
Lysis Buffer SL1	150 mL	2 × 150 mL
Lysis Buffer SL2	150 mL	2 × 150 mL
Lysis Buffer SL3	50 mL	2 × 50 mL
Enhancer SX	50 mL	2 × 50 mL
Binding Buffer SB	250 mL	500 mL
Wash Buffer SW1	125 mL	300 mL
Wash Buffer SW2 (Concentrate) [1]	100 mL	2 × 100 mL
Elution Buffer SE [2]	60 mL	125 mL
MN Bead Tubes	2 × 96	4 × 96
NucleoSpin® Inhibitor Removal Plate (light gray rings)	2	4
NucleoSpin® Soil Binding Plate (light green rings)	2	4
MN Wash Plate [3]	2	4
MN Square-well Block	2	4
Rack of Tube Strips [4]	2	4
User manual	1	1

[1] For preparation of working solutions and storage conditions see section 3.

[2] Composition of Elution Buffer SE: 5 mM Tris/HCl, pH 8.5.

[3] For use with vacuum or positive pressure only.

[4] Set of 1 rack, 12 strips with 8 tubes each, Cap Strips included

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

Reagents

- 96 – 100 % ethanol

For more detailed information regarding special hardware required for centrifuge, vacuum or positive pressure processing, see section 5 or contact Technical Service (tech-bio@mn-net.com).

1.3 About this user manual

It is strongly recommended that first-time users of the **NucleoSpin® 96 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 Plate.

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 Binding Plate.

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® 96 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 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 Beads**.
- The optimized buffer chemistry and the **NucleoSpin® Inhibitor Removal Plate** 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.

The kit can be processed with vacuum, positive pressure or centrifugation, manually or semi automated.

Table 1: Kit specifications at a glance	
Parameter	NucleoSpin® 96 Soil
Technology	Silica membrane technology
Format	96-well plates
Processing	vacuum, positive pressure or centrifugation
Sample material	< 500 mg soil or sediment
Typical yield	2 – 10 µg
Elution volume	100 – 200 µL
Preparation time	150 min/plate
Binding capacity	50 µg
Use	For research use only

2.3 Automated processing on robotic platforms

NucleoSpin® 96 Soil can be processed semi automated on many common laboratory workstations. The bead beating lysis still has to be processed manually but the DNA clean-up can be automated using vacuum or positive pressure for loading, washing and elution. Please contact MN for scripts and general considerations on a specific robot.

The risk of cross-contamination is reduced by optimized vacuum or positive pressure settings, an improved shape of the NucleoSpin® Soil Binding Plate outlets, and application of the MN Wash Plate protecting the bottom and the binding plate outlets from lysate and wash buffer spray. The MN Frame (see ordering information) can be used to position the disposable MN Wash Plate inside the vacuum chamber.

Visit MN online at ***www.mn-net.com*** or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol.

2.4 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.

2.5 Amount of starting material

NucleoSpin® 96 Soil is suitable for processing 250 – 500 mg of sample material. However, do not fill the MN Bead Tube 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.

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).

2.6 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® 96 Soil kit is equipped with two lysis buffers SL1 and SL2 and 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.

After mixing the sample with lysis buffer in the MN Bead Tube, 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), 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.

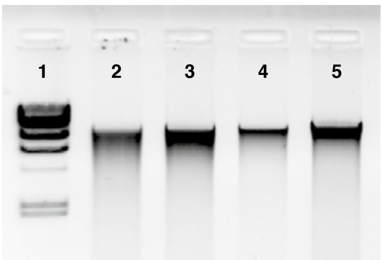


Figure 1 Total DNA purified from wheat field soil with four different lysis buffer combinations
20 of 100 μL eluate were analyzed on a 1 % TAE agarose gel:
Lane 1: Marker λ / HindIII
Lane 2: Lysis Buffer SL1
Lane 3: Lysis Buffer SL1 + Enhancer SX
Lane 4: Lysis Buffer SL2
Lane 5: Lysis Buffer SL2 + Enhancer SX

Table 2: 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

2.7 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 adapter for Vortex-Genie® 2 (MN Bead Tube Holder, see Ordering information, section 6.2). 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.

2.8 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 Lysis Buffer SL3 directly to the MN Bead Tube, 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. Filter both final supernatants of step 4 through a NucleoSpin® Inhibitor Removal Plate 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 Binding Plate according to step 7 in multiple loading steps.

Note that the supplied buffer volumes are calculated for only one extraction per well. The excess of Enhancer SX and Binding Buffer SB might not be sufficient to allow two extraction steps.

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.

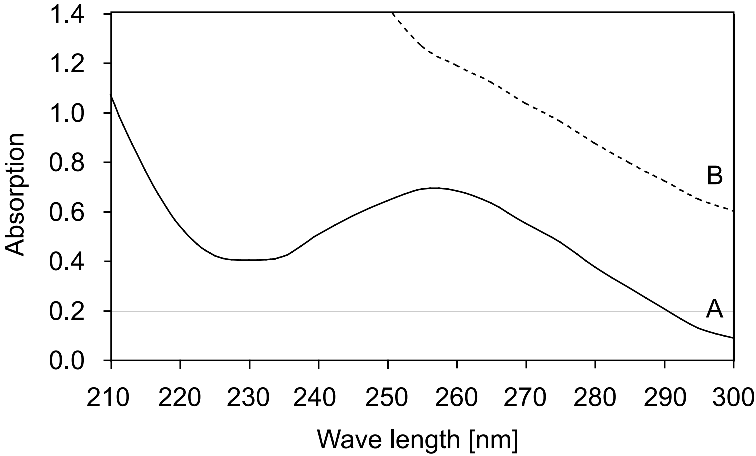


Figure 2 UV-VIS quantification of A) pure DNA and B) contaminated DNA
A) 7.7 µg in 100 µL, 1.84 A260 / A280, 1.71 A260 / A230
B) 9.3 µg in 100 µL, 1.35 A260 / A280, 0.27 A260 / A230

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.

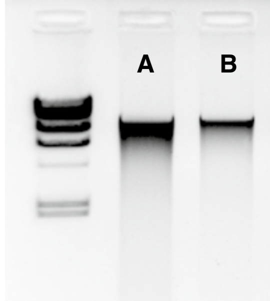


Figure 3 Gel analysis of A) pure and B) contaminated genomic DNA from soil
10 µL of each sample were run on a 1 % TAE agarose gel (1 h, 100 V). The larger gel band of pure DNA A) proves a higher yield and concentration compared to the contaminated DNA sample which is in contrast to the UV-VIS quantification (A: 7.7 µg / 100 µL, B: 9.3 µg / 100 µL).

3 Storage conditions and preparation of working solutions

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

CAUTION: *Buffers SB and SW1 contain guanidinium thiocyanate and guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.*

Storage conditions:

- All kit components should be stored at 15 – 25 °C and are stable for at least one year. 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.

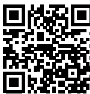
Before starting the first **NucleoSpin® 96 Soil** procedure prepare the following:

- **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.

	NucleoSpin® 96 Soil	
REF	**2 × 96 preps 740787.2**	**4 × 96 preps 740787.4**
Wash Buffer SW2 (Concentrate)	100 mL Add 400 mL ethanol	2 × 100 mL Add 400 mL ethanol to each bottle

4 Safety instructions

When working with the NucleoSpin® 96 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: Guanidinium thiocyanate in Buffer SB and guanidine hydrochloride in 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® 96 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 buffer and treatment 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 NucleoSpin® 96 Soil

5.1 Purification of DNA from soil and sediment – vacuum or positive pressure processing

The NucleoSpin® 96 Soil kit can be used with the NucleoVac 96 Vacuum Manifold (see ordering information). When processing less than 96 samples, Self-adhering PE Foil (see ordering information) should be used to close and protect unused wells to ensure proper vacuum or positive pressure.

Establish a reliable vacuum or positive pressure source for the NucleoVac 96 Vacuum Manifold. It can be used in combination with a vacuum pump, house vacuum, or a water aspirator. We recommend a vacuum of -0.2 to -0.4 bar (reduction of atmospheric pressure) that can be adjusted by the NucleoVac Vacuum Regulator (see ordering information). Alternatively, adjust the vacuum or positive pressure so that sample and buffers run through the plate at a rate of 1 – 2 drops per second.

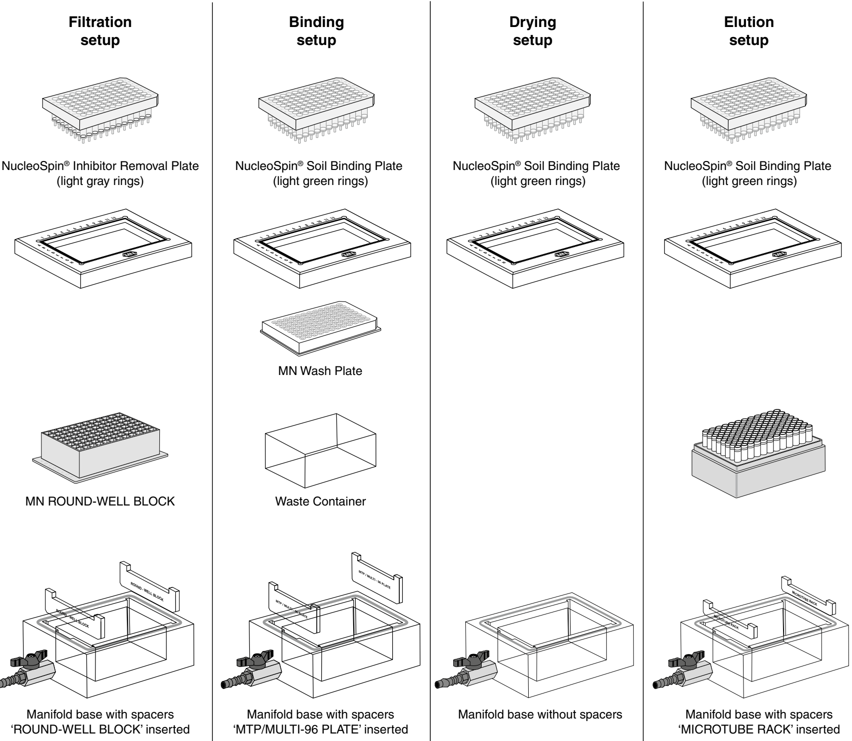
Protocol-at-a-glance

- For detailed information on vacuum manifold setups, see page 17.
- For detailed information on each step, see page 18.
- 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	**250 – 500 mg sample to MN Bead Tube** **700 µL SL1 or SL2**
2	**Adjust** lysis conditions	**150 µL Enhancer SX**
3	**Lyse** sample	**Mechanically homogenize**
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	**Assemble filtration setup** **Load samples -0.7 bar**
6	**Adjust** binding conditions	**250 µL SB** **Mix**

7	**Bind** DNA	**Assemble binding setup** **Load samples** **-0.2 to -0.6 bar**
8	**Wash** silica membrane	**500 µL SB** **-0.2 to -0.6 bar** **550 µL SW1 – 0.2 to -0.6 bar** **700 µL SW2 – 0.2 to -0.6 bar** **700 µL SW2 – 0.2 to -0.6 bar**
9	**Dry** silica membrane	**Assemble drying setup** **Full vacuum, 15 min or 37 °C, 20 min**
10	**Elute** DNA	**Assemble elution setup** **100 – 200 µL SE** **1 min** **-0.2 to -0.4 bar**

Setup of vacuum manifold



Detailed protocol

- 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 2.5 and 2.6 for more information on the amount of starting material and the choice of lysis buffer. See section 2.8 for the repeated extraction of a sample to improve DNA yield.

Transfer **250 – 500 mg** fresh **sample material** to a **MN Bead Tube** 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 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.

Note: Enhancer SX ensures the highest possible DNA yield. It can, however, also promote the release of humic acids. See section 2.6 on how to lower the volume or omit the buffer entirely in order to increase DNA purity.

3 Lyse sample

See section 2.7 for more information on homogenization methods (e.g., FastPrep®-24 instrument, Vortex adapter).

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 2.8 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***.

5 Filter lysate

Assemble filtration setup: Insert spacers labeled 'ROUND-WELL BLOCK' with the notched side up. Place a MN Square-well Block onto the spacers. Close the manifold with the manifold lid and place the NucleoSpin® Inhibitor Removal Plate on top of the lid (see Filtration setup, page 17).

Load up to **1 mL** of **clear supernatant** from step 4 into each well of the plate.

Apply vacuum (-0.7 bar) until all liquid has passed the plate.

Repeat loading step to process more than 1 mL of lysate (see section 2.8 for repeated extraction).

Note: Use centrifuge in case of clogging (minimal bucket height has to be 85 mm to accommodate entire square-well block-filter plate sandwich).

Discard NucleoSpin® Inhibitor Removal Plate.

6 Adjust binding conditions

Add **250 µL Buffer SB** to the flow-through in each well of the MN Square-well Block.

Mix by pipetting up and down.

7 Bind DNA

Assemble binding setup: Place the waste container into vacuum manifold base. Insert spacers labeled 'MTP/MULTI-96 PLATE' with the notched side up. Place the MN Wash Plate onto the spacers. Close the manifold with the manifold lid and place the NucleoSpin® Soil Binding Plate on top of the lid (see Binding setup, page 17).

Load **binding mixtures** from step 6 onto the binding plate.

Apply vacuum (-0.2 to -0.6 bar) until all liquid has passed the plate. Flow-through rate should be about 1 – 2 drops per second. Adjust vacuum strength accordingly.

8 Wash and dry silica membrane

1st wash

Load **500 µL Buffer SB**.

Apply **vacuum (-0.2 to -0.6 bar)** until all liquid has passed the plate.

2nd wash

Load **550 µL Buffer SW1**.

Apply **vacuum (-0.2 to -0.6 bar)** until all liquid has passed the plate.

3rd wash

Load **700 µL Buffer SW2**.

Apply **vacuum (-0.2 to -0.6 bar)** until all liquid has passed the plate.

4th wash

Load **700 µL Buffer SW2**.

Apply **vacuum (-0.2 to -0.6 bar)** until all liquid has passed the plate.

9 Dry silica membrane

Assemble drying setup: Put NucleoSpin® Soil Binding Plate on a clean paper towel to remove residual wash buffer from plate outlets. Discard MN Wash Plate and remove waste container from vacuum manifold. Close the manifold with the manifold lid and place the NucleoSpin® Soil Binding Plate back on top of the lid (see Drying setup, page 17).

Apply **maximum vacuum** for **15 min** to dry membrane and to eliminate last traces of ethanol.

Note: Ethanol in Buffer SW2 inhibits enzymatic reactions and has to be removed completely before eluting the DNA. An additional 10 min incubation of the binding plate at 37 °C can further improve ethanol removal.

10 Elute DNA

Assemble elution setup: Insert spacers 'MICROTUBE RACK' into the vacuum manifold base. Place the Rack of Tube Strips onto the spacers. Close the manifold with the manifold lid and place the NucleoSpin® Soil Binding Plate back on top of the lid (see Elution Setup on page 17).

Load **100 – 200 μL Buffer SE** directly into the center of the silica membrane of each well.

Incubate for **1 min**.

Apply **vacuum (-0.2 to -0.4 bar)** until all liquid has passed the plate.

Note: Preheating Buffer SE to 70 °C or / and incubating the entire plate at elevated temperature for 5 min can increase final yield significantly. Furthermore, two consecutive elution steps (e.g., 2 × 100 μL) yield more DNA than one elution step with 200 μL.

Check DNA yield and quality by UV-VIS and agarose gel (see section 2.9 for more details).

5.2 Purification of DNA from soil and sediment – centrifuge processing

For this protocol a microtiter plate centrifuge is required that is able to accommodate the NucleoSpin® Soil Binding Plate stacked on the MN Square-well Block (minimum bucket height 85 mm). The centrifuge should be able to reach accelerations of 5,600 – 6,000 x *g*.

To collect flow-throughs in step 7 and 8, additional MN Square-well or Round-well Blocks are required (see ordering information). They can be recycled by washing with detergent and hot water. Incubate for 5 min in 0.4 M HCl, rinse with water again, and autoclave before next use.

Protocol-at-a-glance

- For detailed information on each step, see page 24.
- 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	**250 – 500 mg sample to MN Bead Tube** **700 µL SL1 or SL2**
2	**Adjust** lysis conditions	**150 µL Enhancer SX**
3	**Lyse** sample	**Mechanically homogenize**
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	**Assemble filtration setup** **Load samples** **5,600 – 6,000 x *g*, 5 min**
6	**Adjust** binding conditions	**250 µL SB** **Mix**
7	**Bind** DNA	**Load samples** **5,600 – 6,000 x *g*, 5 min**

8	**Wash** silica membrane	**500 µL SB** **5,600 – 6,000 x *g*, 5 min** **550 µL SW1** **5,600 – 6,000 x *g*, 2 min** **700 µL SW2** **5,600 – 6,000 x *g*, 2 min** **700 µL SW2** **5,600 – 6,000 x *g*, 5 min**
9	**Dry** silica membrane	**5,600 – 6,000 x *g*, 15 min** **or** **37 °C, 20 min**
10	**Elute** DNA	**100 – 200 µL SE** **1 min** **5,600 – 6,000 x *g*, 2 min**

Detailed protocol

- 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 2.5 and 2.6 for more information on the amount of starting material and the choice of lysis buffer. See section 2.8 for the repeated extraction of a sample to improve DNA yield.

Transfer **250 – 500 mg** fresh **sample material** to a **MN Bead Tube** 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 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.

Note: *Enhancer SX ensures the highest possible DNA yield. It can, however, also promote the release of humic acids. See section 2.6 on how to lower the volume or omit the buffer entirely in order to increase DNA purity.*

3 Lyse sample

See section 2.7 for more information on homogenization methods (e.g., FastPrep®-24 instrument, Vortex adapter).

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 2.8 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*.

5 Filter lysate

Place the NucleoSpin® Inhibitor Removal Plate onto the **MN Square-well Block**.

Load up to 1 mL **clear supernatant** from step 4 to each well of the plate.

Centrifuge at **5,600 – 6,000 x *g*** for 5 min.

Repeat loading step to process more than 1 mL of lysate (see section 2.8 for repeated extraction).

Discard NucleoSpin® Inhibitor Removal Plate.

6 Adjust binding conditions

Add **250 µL Buffer SB** to the flow-through in each well of the MN Square-well Block.

Mix by pipetting up and down.

7 Bind DNA

Place the **NucleoSpin® Soil Binding Plate** on top of a **new square-well or round-well block** (not provided).

Load **binding mixtures** from step 6 onto the binding plate.

Centrifuge at **5,600 – 6,000 x *g*** for 5 min. Discard flow-through.

8 Wash and dry silica membrane

1st wash

Load **500 µL Buffer SB**.

Centrifuge at **5,600 – 6,000 x *g*** for **2 min**. Discard flow-through.

2nd wash

Load **550 µL Buffer SW1**.

Centrifuge at **5,600 – 6,000 x *g*** for **2 min**. Discard flow-through.

3rd wash

Load **700 µL Buffer SW2**.

Centrifuge at **5,600 – 6,000 x *g*** for **2 min**. Discard flow-through.

4th wash

Load **700 µL Buffer SW2**.

Centrifuge at **5,600 – 6,000 x *g*** for **5 min**. Discard flow-through.

9 Dry silica membrane

Centrifuge at **5,600 – 6,000 x *g*** for **15 min** or incubate the plate for **20 min** at **37 °C**.

Note: Ethanol in Buffer SW2 inhibits enzymatic reactions and has to be removed completely before eluting the DNA. Incubation at 37 °C is more effective to remove traces of wash buffer and should be preferred if possible.

10 Elute DNA

Place the NucleoSpin® Soil Binding Plate on an opened Rack of Tube Strips.

Load **100 – 200 µL Buffer SE** directly into the center of the silica membrane of each well.

Incubate for **1 min**.

Centrifuge at **5,600 – 6,000 x *g*** for **2 min**.

Note: Preheating Buffer SE to 70 °C or / and incubating the entire plate at elevated temperature for 5 min can increase final yield significantly. Furthermore, two consecutive elution steps (e.g., 2 × 100 µL) yield more DNA than one elution step with 200 µL.

Check DNA yield and quality by UV-VIS and agarose gel (see section 2.9 for more details).

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. Too little head space does not allow the necessary motion of the beads to disrupt the sample. Use less sample material (see section 2.5 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 2.6 for more information). • *Insufficient disruption and / or homogenization of starting material* • Shaking of the MN Bead Tube 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 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 Binding Plate completely before elution to avoid carry-over of ethanolic Wash Buffer SW2. *Contamination with PCR inhibitors* • The DNA purity can be increased by lowering the amount of starting material (see section 2.5 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 2.6 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® 96 Soil	740787.2 740787.4	2 × 96 preps 4 × 96 preps
Buffer SB	740785.50	50 mL
Buffer SL1	740781.30	30 mL
Buffer SL2	740782.30	30 mL
Buffer SL3	740783.30	30 mL
Enhancer SX	740784.50	50 mL
MN Bead Tubes Type A (0.6 – 0.8 mm ceramic beads; recommended for stool, soil, and sediments)	740786.50	50

Product	REF	Pack of
MN Bead Tubes Type B (40 – 400 μm glass beads; recommended for bacteria)	740812.50	50
MN Bead Tubes Type C (1 – 3 mm corundum; recommended for yeast)	740813.50	50
MN Bead Tubes Type D (3 mm steel balls; recommended for insects)	740814.50	50
MN Bead Tubes Type E (40 – 400 μm glass beads and 3 mm steel balls; recommended for hard-tolyse bacteria within insect or tissue samples)	740815.50	50
MN Bead Tubes Type F (1 – 3 mm corundum + 3 mm steel balls; use only with MN Bead Tube Holder!)	740816.50	50
MN Bead Tube Holder	740469	1
MN Square-well Block	740476 740476.24	4 24
MN Wash Plate	740479 740479.24	4 24
Rack of Tube Strips (1 set consists of 1 rack, 12 strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 24
Cap Strips	740478 740478.24	48 288
Self-adhering PE Foil	740676	50
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1

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

6.3 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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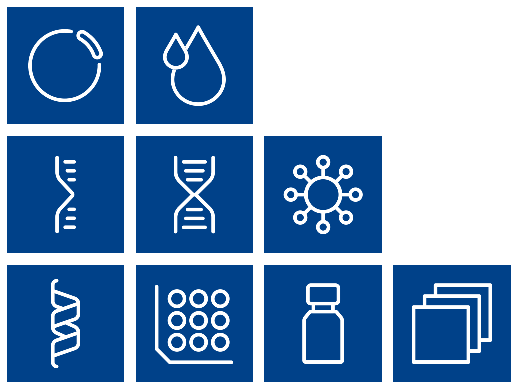
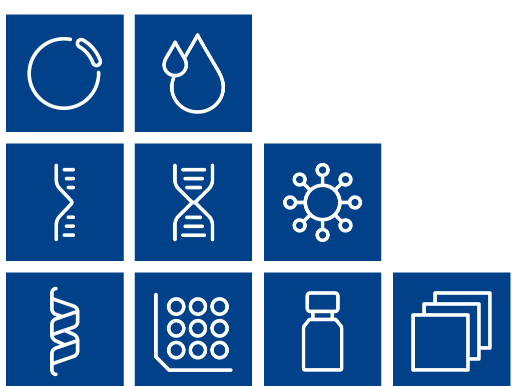


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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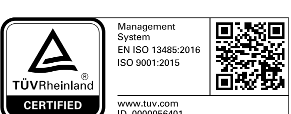

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