Genomic DNA from soil

User manual

NucleoSpin® Soil

November 2011 / Rev. 03

MACHEREY-NAGEL

Genomic DNA from soil

Protocol-at-a-glance (Rev. 03)

		NucleoSpin® Soil
1	**Prepare sample**	 NucleoSpin® Bead Tube 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 700 µL SW2 Vortex 2 s 11,000 x *g*, 30 s 4th 700 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

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-270 · Fax: +49 24 21 969-199 · tech-bio@mn-net.com · ***www.mn-net.com***

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1 Components

1.1 Kit contents

	NucleoSpin® Soil		
	10 preps	50 preps	250 preps
REF	740780.10	740780.50	740780.250
Lysis Buffer SL1	30 mL	2 x 30 mL	250 mL
Lysis Buffer SL2	30 mL	2 x 30 mL	250 mL
Lysis Buffer SL3	5 mL	15 mL	50 mL
Enhancer SX	3 mL	10 mL	50 mL
Binding Buffer SB	10 mL	2 x 25 mL	2 x 125 mL
Wash Buffer SW1	6 mL	30 mL	2 x 75 mL
Wash Buffer SW2 (Concentrate)*	6 mL	20 mL	2 x 50 mL
Elution Buffer SE**	5 mL	15 mL	30 mL
NucleoSpin® Bead Tubes	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
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* 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

- 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 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 **NucleoSpin® Beads**.
- 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

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 NucleoSpin® 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 NucleoSpin® 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).

2.5 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.

After mixing the sample with lysis buffer in the NucleoSpin® 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), 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.

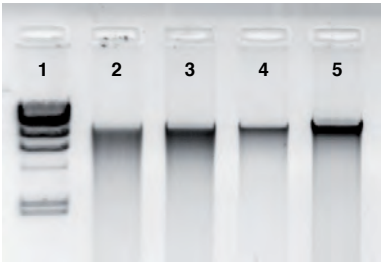

Figure 1: Total DNA purified from wheat field soil with four different lysis buffer combinations

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

Lane 1: Marker λ / *Hin*dIII

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

2.6 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 adapter for Vortex-Genie® 2 (MO BIO). 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 NucleoSpin® 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 NucleoSpin® Bead Tube. 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 Bindung Buffer SB might not be sufficient to allow two extraction steps for all 10, 50, or 250 preps of the kit.

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 230nm. 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.

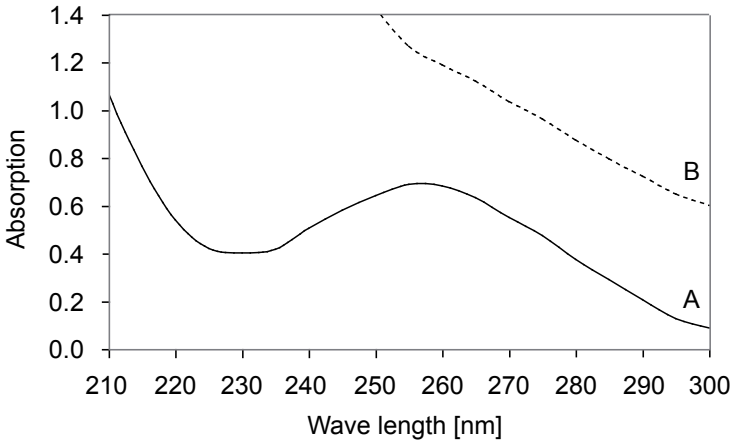

Figure 2: UV-VIS quantification of A) pure DNA and B) contaminated DNA

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.

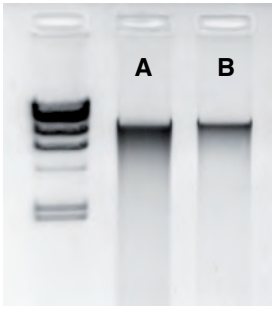

Figure 3: Gel analysis of A) pure and B) contaminated genomic DNA from soil

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).

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 room temperature (18–25 °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® 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 room temperature (18–25 °C) for at least one year.

	NucleoSpin® Soil		
	10 preps	**50 preps**	**250 preps**
REF	**740780.10**	**740780.50**	**740780.250**
Wash Buffer SW2 (Concentrate)	6 mL Add 24 mL ethanol	20 mL Add 80 mL ethanol	2 x 50 mL Add 200 mL ethanol to each bottle

4 Safety instructions

The following components of the **NucleoSpin® Soil** kits contain hazardous contents. *Wear gloves and goggles and follow the safety instructions given in this section.*

4.1 Risk and safety phrases

Component *Inhalt*	Hazard contents *Gefahrstoff*	Hazard symbol *Gefahrstoff-symbol*	Risk phrases *R-Sätze*	Safety phrases *S-Sätze*
SB	Guanidine thiocyanate 30–60 % *Guanidiniumthiocyanat 30–60%*	Xn*	R 20/21/22-32-52/53	S 13-61
SW1	Guanidine hydrochloride 36–50 % + isolpropanol 20–50 % *Guanidinhydrochlorid 36–50 % + Isopropanol 20–50 %*	Xn*	R 10-22-36	S 16-26-39

Risk phrases

R 10	Flammable. *Entzündlich.*
R 20/21/22	Harmful by inhalation, in contact with the skin, and if swallowed. *Gesundheitsschädlich beim Einatmen, Verschlucken und Bwrührung mit der Haut..*
R 22	Harmful if swallowed. *Gesundheitsschädlich beim Verschlucken.*
R 32	Contact with acids liberates very toxic gas. *Entwickelt bei Berührung mit Säure sehr giftige Gase.*
R 36	Irritating to eyes. *Reizt die Augen.*
R 52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. *Schädlich für Wasserorganismen, kann in Gewässern lämgerfristig schädliche Wirkungen haben.*

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

Safety phrases

S 13	Keep away from food, drink, and animal foodstuffs. *Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.*
S 16	Keep away from sources of ignition - No smoking. *Von Zündquellen fernhalten – Nicht rauchen.*
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. *Bei Berührung mit den Augen gründlich mit Wasser abspülen und Arzt konsultieren.*
S 39	Wear eye / face protection. *Schutzbrille / Gesichtsschutz tragen.*
S 61	Avoid release to the environment. Refer to special instructions/safety data sheet. *Bei Berührung mit den Augen sofort gründlich mit Wasser abspülen und Arzt konsultieren.*

4.2 GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
SB	Guanidine thiocyanate 30–60 % *Guanidiniumthiocyanat 30–60%*	 Warning *Achtung*	302, 412, EUH 031	260, 273, 301+312, 330
SW1	Guanidine hydrochloride 36–50 % + isolpropanol 20–50 % *Guanidinhydrochlorid 36–50 % + Isopropanol 20–50 %*	 Warning *Achtung*	226, 302, 319	210, 233, 280, 301+312, 305+351+338, 330, 337+313, 403+235

Hazard phrases

H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*
H 412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
EUH 031	Contact with acids liberates toxic gas. *Entwickelt bei erührung mit Säure giftige Gase.*

Precaution phrases

P 210	Keep away from heat / sparks / open flames / hot surfaces – No smoking. *Von Hitze / Funken / offener Flamme / heißen Oberflächen fernhalten – Nicht rauchen.*
P 233	Keep container tightly closed. *Behälter dicht verschlossen halten.*
P 260	Do not breathe vapours. *Dampf nicht einatmen.*
P 273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P 280	Wear protective gloves / eye protection. *Schutzhandschuhe / Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER or doctor /physician if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM oder Arzt anrufen.*
P 305+351+338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. *BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.*
P 330	Rinse mouth. *Mund ausspülen.*
P 337+313	Get medical advice / attention. *Bei anhaltender Augenreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 403+235	Store in a well ventilated place. Keep container tightly closed. *Kühl an einem gut belüfteten Ort aufbewahren.*

For further information please see Material Safety Data Sheets ***(www.mn-net.com).***
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ***(www.mn-net.com)****.*

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.

Transfer **250–500 mg** fresh **sample material** to a **NucleoSpin® Bead Tube** containing the ceramic beads.

250–500 mg sample

+ 700 µL SL1 or SL2

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 NucleoSpin® 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.

+ 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).

Attach the NucleoSpin® 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**.

Vortex RT, 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

+ 250 µL SB

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

Vortex 5 s

Vortex for **5 s**.

7 Bind DNA

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

Load 550 µL sample

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

11,000 x *g*, 1 min

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

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

Load remaining sample

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

11,000 x *g*, 1 min

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

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

8 Wash and dry silica membrane

1st wash

+ 500 µL SB

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

11,000 x *g*, 30 s

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

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

2nd wash

+ 550 µL SW1

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

11,000 x *g*, 30 s

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

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

3rd wash

+ 700 µL SW2

Add **700 µL Buffer SW2** to the NucleoSpin® Soil Column.

Vortex 2 s

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

Add **700 µL Buffer SW2** to the NucleoSpin® Soil Column.

+ 700 µL SW2

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.

Vortex 2 s

11,000 x *g*, 30 s

Note: 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.

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9 Dry silica membrane

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

11,000 x *g*, 2 min

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.

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10 Elute DNA

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

30–100 µL SE

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

RT, 1 min

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***.

11,000 x *g*, 30 s

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).

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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 NucleoSpin® 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 2.4 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 NucleoSpin® 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 NucleoSpin® 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 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
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
NucleoSpin® Bead Tubes	740786.50	50
Collection Tubes (2 mL)	740600	1000

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

6.3 Product use restriction/warranty

NucleoSpin® Soil kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN VITRO*-diagnostic use. Please pay attention to the package of the product. *IN VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-270
tech-bio@mn-net.com