GENETIX BRAND

HANDBOOK

Nucleo-pore®

Nucleo-pore® gDNA Soil Kit

☐ **Nucleo-pore® gDNA Soil Kit**	NP-1006D	50 Preps
☐ **Nucleo-pore® gDNA Soil Kit**	NP-1007D	250 Preps

www.genetixbiotech.com

Nucleo-pore®

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COMPONENTS

Kit contents

Nucleopore® gDNA Soil Kit

Cat #	NP-1006D	NP-1007D
No. of Preps	50	250
Lysis Buffer SL1	60ml	250ml
Lysis Buffer SL2	60ml	250ml
Lysis Buffer SL3	10ml	50ml
Enhancer SX	10ml	50ml
Binding Buffer SB	60ml	250ml
Wash Buffer SWB1	30ml	150ml
Wash Buffer SWB2*	25ml	100ml
Elution Buffer SEB**	13ml	60ml
NP Bead Tubes Type A	50	250
NP Inhibitor Removal Columns (Red Rings)	50	250
NP Soil Columns (Green Rings)	50	250
Collection Tube (2ml)	50	250
Collection Tube (2ml, lid)	50	250
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*Please see “Preparation and Storage of reagents“

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

Reagents, Consumables and equipment not provided with the kit

- 96 – 100% ethanol
- Beta-mercaptoethanol to 0.5%(v/v)
- 1.5 ml microcentrifuge tubes
- Disposable pipette tips
- Manual pipetts
- Centrifuge
- Vortex
- Equipment for sample disruption and homogenization
- Personal protection equipment (e.g., lab coat, gloves, goggles

SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS).

Caution: Guanidin thiocyanate in Buffer SB and buffer SWB1 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 Nucleo-pore® dDNA 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.

Disposal

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

INTRODUCTION

Principle and Procedure

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 Nucleo-pore®
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 Nucleo-pore® Soil Column.

Residual humic substances, especially humic acids, and other PCR inhibitors are removed by efficient washing with Binding Buffer SB and Wash Buffers SWB1/SWB2. After a drying step, ready to use DNA can be eluted with Elution Buffer SEB (5 mM Tris/HCl, pH 8.5).

Specifications of Nucleo-pore® gDNA Soil Kit

- The Nucleo-pore® 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 NP Bead Tubes Type A.
- The optimized buffer chemistry and the Nucleo-pore® 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.

Kit specifications at a glance

Parameter	Nucleo-pore® 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

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.

Amount of starting material

Nucleo-pore® Soil is suitable for processing 250 – 500 mg of sample material. However, do not fill the NP 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 NP.

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).

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 Nucleo-pore® 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 NP 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 (A260/A230) of the sample significantly, might, however, lower the DNA yield. 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.

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, or an NP 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 should be avoided to minimize liberation of humic acids.

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. But instead of adding SL3 directly to the NP 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 NP Bead Tube Type A. Filter both final supernatants of step 4 through a Nucleo-pore® Inhibitor Removal Column. Add Binding Buffer SB to both filtrates according to step 6 and finally load both samples on one Nucleo-pore® 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.

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.

Preparation & Storage of Reagents

Buffers SB and SWB1 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 Nucleo-pore® Soil procedure prepare the following:

- **Wash Buffer SWB2**: Add the indicated volume of ethanol (96 – 100 %) to Buffer SWB2 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Buffer SWB2 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

Nucleo-pore® gDNA Soil Kit		
Cat. No.	50 preps NP-1006D	250 preps NP-1007D
Wash Buffer SWB2 (Concentrate)	25 mL Add 100 mL ethanol	100 mL Add 400 mL ethanol

PROTOCOL

Genomic DNA Purification 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.

Prepare sample

250 – 500 mg sample
+700 µL SL1 or SL2

Transfer 250 – 500 mg fresh sample material to a NP 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 NP 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.

Adjust lysis conditions

+150 µL SX

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.

Sample lysis

Vortex
RT, 5 min

Attach the NP 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.

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

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.

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.

Filter lysate

Place a Nucleo-pore® 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 Nucleo-pore® Inhibitor Removal Column to a new collection tube (not provided) and load the remaining supernatant. Centrifuge for 1 min at 11,000x g. Combine the flow throughs.

Discard the Nucleo-pore® 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

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

Bind DNA

Place a Nucleo-pore® Soil Column (green ring) in a Collection Tube (2mL).
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 th 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

Wash and dry silica membrane

1st wash

Add 500 µL Buffer SB to the Nucleo-pore® Soil Column.

Centrifuge for 30 s at 11,000 x g.

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

+500 µL SB

11,000 x g, 30 s

2nd wash

Add 550 µL Buffer SWB1 to the Nucleo-pore® Soil Column.

Centrifuge for 30 s at 11,000 x g.

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

+550 µL SWB1

11,000 x g, 30 s

3rd wash

Add 650 µL Buffer SWB2 to the Nucleo-pore® 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.

+650 µL SWB2

Vortex 2 s

11,000 x g, 30 s

4th wash

Add 650 µL Buffer SWB2 to the Nucleo-pore® 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.

Note: The same collection tube is used throughout the entire washing procedure to reduce plastic waste.

+650 µL SWB2

Vortex 2 s

11,000 x g, 30 s

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 Nucleo-pore® Soil Column after the drying step, discard flow through and centrifuge again.

11,000 x g, 2 min

Elute DNA

Place the Nucleo-pore® 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 SEB 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.

30 – 100µL SE

RT, 1 min

11,000 x g,

TROUBLESHOOTING GUIDE

Poor quality DNA

Possible cause

- Buffers and Reagents not re-constituted properly

Suggestions

- Reconstitute buffers solution as per instructions.

Possible cause

- Insufficient cell lysis

Suggestions

- Vortex the mixture vigorously immediately after addition of Lysis Buffer SL1.

Possible cause

- RNA Contamination

Suggestions

- Add 20ul RNase A solution (20 mg/ml) before addition of Lysis Buffer SL1 if RNA free DNA is required.

Possible cause

- Samples inappropriate.

Suggestions

- Use recommended amount of starting material

Suboptimal performance of genomic DNA in enzymatic reactions

Possible cause

- Ethanol not completely removed

Suggestions

- Make sure to remove all of ethanol before eluting the DNA. If required add a dry spin after second wash with buffer SWB2 at 10,000 x g for 3 minutes

Possible cause

- Co-purification of inhibitory substances

Suggestions

- Use EDTA free elution buffer. It is recommended to use the Elution Buffer SEB provided with the kit.

TROUBLESHOOTING GUIDE

Low DNA yield

Possible cause

- Inefficient homogenization of samples

Suggestions

- Repeat protocol using new samples and ensure complete homogenization

Possible cause

- Inefficient cell lysis due to insufficient mixing of the sample with Lysis Buffer Sl1

Suggestions

- Repeat the DNA purification procedure with a new sample. Vortex the mixture vigorously immediately after addition of Lysis Buffer Sl1.

Possible cause

- Suboptimal elution of DNA from the column

Suggestions

- Preheat Elution Buffer SEB to 70 °C before elution. Apply Elution Buffer SEB directly onto the center of the silica membrane. Check the pH of Elution Buffer SEB as elution efficiency decreases dramatically if elution is performed with buffers of pH < 7.0. It is always recommended to use the Elution Buffer SEB supplied with the kit.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
° DNASure Tissue Mini Kit	50 preps	NP-61305
° DNASure Plant Mini Kit	50 preps	NP-79105
° DNASure Plant Mini Kit	250 preps	NP-79107
° DNASure Plant Midi Kit	20 preps	NP-78153
° DNASure Plant Maxi Kit	10 preps	NP-78164
° DNASure Blood Mini Kit	50 preps	NP-61105
° DNASure Blood Mini Kit	250 preps	NP-61107
° DNASure Blood Midi Kit	20 preps	NP-61184
° DNASure Blood Maxi Kit	10 preps	NP-61193
° DNASure Blood FastPure Kit	50 preps	NP-62205
° DNASure Blood FastPure Kit	250 preps	NP-62207
° SureSpin Plasmid Mini Kit	50 preps	NP-37105
° SureSpin Plasmid Mini Kit	250 preps	NP-37107
° SureSpin Plasmid FastPrep Kit	50 preps	NP-47105
° SureSpin Plasmid FastPrep Kit	250 preps	NP-47107
° SureSpin Buffer Set*	1	37107-BS
° SurePrep Plasmid Mini Kit	20 preps	NP-15123
° SurePrep Plasmid Mini Kit	100 preps	NP-15125
° SurePrep Plasmid Midi Kit	20 preps	NP-15143
° SurePrep Plasmid Midi Kit	100 preps	NP-15145
° SurePrep Plasmid Maxi Kit	10 preps	NP-15161
° SurePrep Plasmid Maxi Kit	25 preps	NP-15162
° SurePrep Plasmid Mega Kit	5 preps	NP-15183
° SurePrep Plasmid Giga Kit	5 preps	NP-15191

***SureSpin° Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

Nucleopore® gDNA Soil Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the Nucleopore® gDNA Soil Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of Nucleopore® gDNA Soil Kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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