



GENETIX BRAND

HANDBOOK



## Purification of DNA from Soil

Nucleo-pore gDNA Soil Kit      NP-1006D      50 Preps



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## COMPONENTS

### Kit contents

#### Nucleopore® gDNA Soil Kit

Cat #	NP-1006D
No. of Preps	50
Lysis Buffer SL	40ml
Binding Buffer SB	100ml
Pre- Wash Buffer SPW*	15 ml
Wash Buffer SWB*	50 ml
Elution Buffer SEB	10ml
Thrashing Bead Lysis tube	50
Fast Spin Filter	50
Post Elution Filter	50
Fast Spin Column	50
Collection Tube	200
Handbook	1

\*Please see "Preparation and Storage of reagents"

### 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 pipettes
- 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).

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## INTRODUCTION

### Principle and Procedure

Nucleo-pore gDNA Soil Kit is a simple & fast bead-beating method designed to isolate high quality genomic DNA from a microbes in soil sample sources from clay, sandy, salty, silty, peat, chalky, and loamy soils etc. The kit provides pure gDNA free from PCR inhibitors. Bacteria, fungi, algae, protozoa, etc. can be isolated from upto 0.25g of soil or from fungal/bacterial cells directly. The soil samples are directly added to a Thrashing Bead Lysis Tube and rapidly lysed by bead beating in a vortex without the use of organic denaturants or proteinases. The cellular components are lysed mechanically and DNA from lysed cells binds to a silica matrix. The DNA is recovered by rehydration with elution buffer. The isolated and purified DNA is ideal for downstream molecularbased applications including PCR, arrays, genotyping, methylation profiling etc.

### Specifications of Nucleo-pore® gDNA Soil Kit

The kit is capable of recovering genomic DNA up to and above 40 kb. In most instances, mitochondrial DNA and viral DNA (if present) will also be recovered. Upto 25µg of pure DNA can be purified with an A260/A280 > 1.8 using upto 0.25g sample in an elution volume of 100µl (25 ul minimum).

### Preparation & Storage of Reagents

Kit can be used for up to one year from date of purchase. Every lot of the kit is tested to ensure consistent and high quality results.

### Binding Buffer SB

For optimal performance, add beta-mercaptoethanol (not provided with the kit) to the Binding Buffer SB to a final dilution of 0.5%(v/v) i.e., 500 µl per 100 ml.

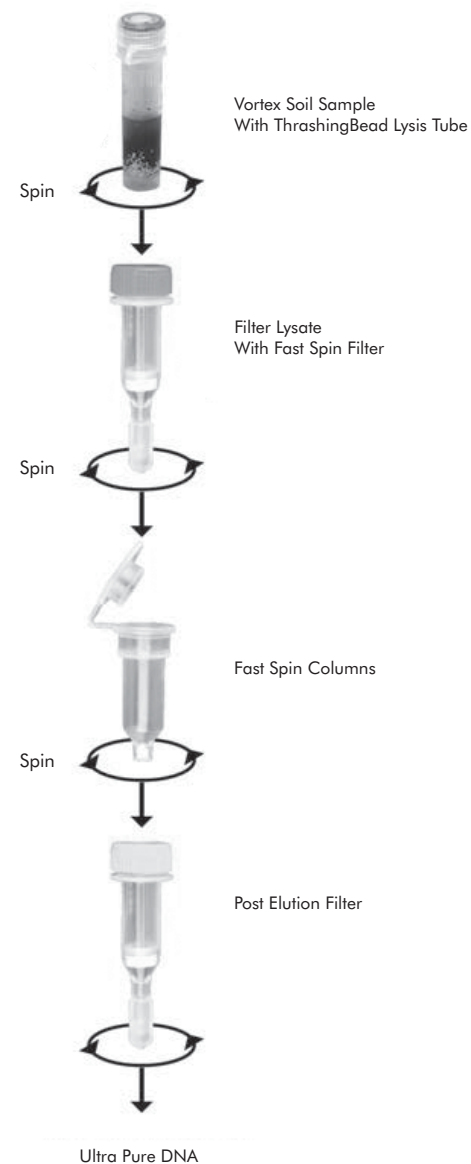
### Pre-Wash Buffer SPW

A precipitate may have formed in the Pre-Wash Buffer SWB during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37°C for 30 minutes and mix by inversion. DO NOT MICROWAVE.

### Preparation of Post Elution Filter

- Gently snap off the base of the filter,
- Insert it into a Collection Tube
- Spin in a micro centrifuge at 8,000 x g for 3 minutes.

## Genomic DNA Purification from Soil



## PROTOCOL FOR GENOMIC DNA PURIFICATION FROM SOIL SAMPLES

### Things to do before starting

- Check whether BME was added to the Binding Buffer SB as per instructions
- Check if Post Elution Filter was prepared as per instructions

Note: if the Fast Spin matrix is dry, add 400-600 µl water prior to prepping the filter.

### PROCEDURE

- 1. Take up to 0.25 grams of soil sample in a fresh Thrashing Bead Lysis Tube (provided), to it add 750 µl Lysis Buffer SL. Ensure the lid is tightly closed in order to prevent leakage.**
- 2. Secure the Thrashing Bead Lysis tube in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 5 minutes.**  
Note: Processing time may be as little as 40 seconds when using high speed cell disrupters. Alternatively, a standard bench top vortex can be used although the overall DNA yield may be lower.
- 3. Centrifuge the Thrashing Bead Lysis Tube in a microcentrifuge at  $\geq 10,000$  x g for 1 minute.**
- 4. Transfer 400µl supernatant to a Fast Spin Filter placed in a collection Tube and centrifuge at 7,000 x g for 1 minute.**  
Note: Ensure to snap off the base of the Fast Spin Filter prior to use.
- 5. Add 1,200 µl of Binding Buffer SB to the filtrate in the Collection Tube from Step 4.**
- 6. Transfer 800 µl of the mixture from Step 5 to Fast Spin Columns in a collection Tube and centrifuge at 10,000 x g for 1 minute.**  
Note: The loading capacity of Fast Spin Columns is 800 µl.
- 7. Discard the flow through from the collection tube and repeat Step 6 with the remaining mixture from Step 5.**
- 8. Add 200 µl Pre-Wash Buffer SPW to the Fast Spin Columns in a new collection tube and centrifuge at 10,000 x g for 1 minute.**
- 9. Add 500 µl Wash Buffer SWB to the Fast Spin Columns and centrifuge at 10,000 x g for 1 minute.**

- 10. Transfer the Fast Spin Columns to a clean 1.5 ml microcentrifuge tube and add 100 µl (25 µl minimum) Elution Buffer SEB directly to the column avoid wetting the rim. Allow it to stand for a minute then centrifuge at 10,000 x g for 30 seconds to elute the DNA.**
- 11. Transfer the eluted DNA from Step 10 to a prepared Post Elution Filter in a clean 1.5 ml microcentrifuge tube and centrifuge at 8,000 x g for 1 minute.**  
Note: Ensure that the Post Elution Filter has already been prepared as per instructions
- 12. The purified DNA is now suitable for downstream applications.**

## 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 SL.

#### Possible cause

- RNA Contamination

#### Suggestions

- Add 20ul RNase A solution (20 mg/ml) before addition of Lysis Buffer SL 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 Step 9 at 10,000 xg 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 SL

#### Suggestions

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

#### 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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