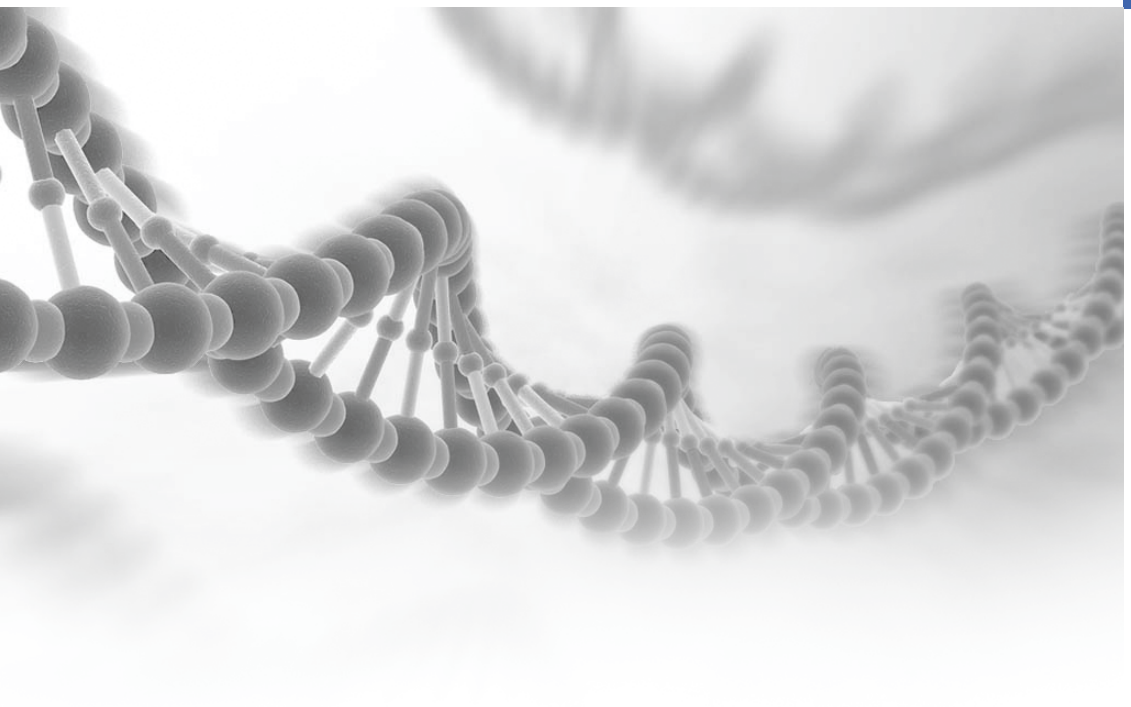
GENETIX BRAND

HANDBOOK

Nucleo-pore®



Nucleo-pore® Stool DNA Mini Kit

☐	**Nucleo-pore® Stool DNA Mini Kit**	NP-7011D	50 Preps

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Nucleo-pore®

Table of Content

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COMPONENTS

Kit contents

Nucleo-pore® Stool DNA Mini Kit

Cat. No.	NP-7011D
Number of Preps	**50 preps**
Lysis Buffer FL	40 ml
Binding Buffer*FB	100 ml
Pre-Wash Buffer* FPW	15 ml
Wash Buffer FWB	50 ml
Elution Buffer FEB	10 ml
Thrashing Bead Lysis Tubes	50
FastSpin Filter	50
Post Elution Filter	50
Fast Spin Columns	50
Collection Tubes	200
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* Please see “Preparation 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 pipetts
- Centrifuge
- Vortex
- Equipment for sample disruption and homogenization
- Personal protection equipment (e.g., lab coat, gloves, goggles)

SAFETY INSTRUCTIONS

Always wear chemical resistant gloves, and safety goggles/face-mask/face shield when working with chemicals. Do not inhale or breathe vapor. Do not get into contact with eyes, skin and clothing. Avoid prolonged or repeated exposure. Keep reagents away from heat and open flame. Store in a cool dry place. Wash your hands thoroughly after handling reagents.

Lysis Solution
Contain Tris (hydroxymethyl) aminomethane (Component 1) and Sodium Chloride (Component 2) S 24/25

DNA Binding Buffer
Contain Guanidine Thiocyanate (Component 1) R 20/21/22, R 32, S 22, S 24-25, S 45; Contain beta mercaptoethanol(Component 2): R27,R25,R34,R51S36/37/39, S45

DNA Wash Buffer FWB
Contain Guanidine Hydrochloride R36, R67, S7, S16, S24/25, S26

R20/21/22: Harmful by inhalation, in contact with skin and if swallowed, R25: Toxic if swallowed, R27: Very toxic in contact with skin, R25: Toxic if swallowed , R32: Contact with acids liberates very toxic gas, R34: Causes burns, R36- Irritating to eyes, R51: Toxic to aquatic organisms, R67- Vapors may cause drowsiness and dizziness, S7- Keep container tightly closed, S16- Keep away from sources of ignition – No smoking , S24/25- Avoid contact with skin and eyes , S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice , S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, 36/37/39: Wear suitable protective clothing, gloves and eye/face protection, S45: In case of accident or if you feel unwell seek medical advice immediately.

INTRODUCTION

Principle and Procedure

Nucleo-pore® Stool DNA Mini Kit is a simple & fast bead-beating method designed to isolate high quality genomic DNA from a variety of stool sample sources from humans, birds, rats, mice, cattle, etc. The kit provides pure gDNA free from PCR inhibitors. Bacterial, Protist as well as host DNA can be efficevtively isolated from ≤ 150 mg sample of mammalian stool with a user friendly protocol. The stool 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 molecular-based applications including PCR, arrays, genotyping, methylation profiling etc.

Specifications of Nucleo-pore® Stool DNA Mini 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 between 1.7 and 1.9 using upto 150mg sample in an elution volume of 100µl.

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

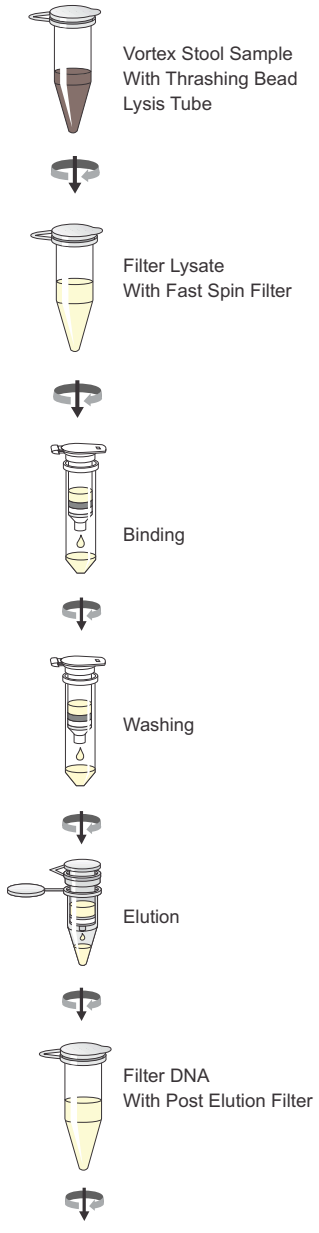
For optimal performance, add beta-mercaptoethanol (not provided with the kit) to the Binding Buffer FB to a final dilution of 0.5%(v/v) i.e., 500 µl per 100 ml.

Pre-Wash Buffer

A precipitate may have formed in the Pre-Wash Buffer FWB during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 ºC 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 microcentrifuge at 8,000 x g for 3 minutes.



Protocol for DNA purification from Human or animal stool

Things to do before starting

- Check whether BME was added to the Binding Buffer FB 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 150 mg of Stool sample in a fresh Thrashing Bead Lysis Tube (provided), to it add 750 µl Lysis Buffer FL. 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 ≥ 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 FB to the filtrate in the Collection Tube from Step 4.**

6. **Transfer 800 µl of the mixture from Step 5 to a 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 FPW 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 FWB 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 Elution Buffer FEB 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 FL.

Possible cause
- RNA Contamination

Suggestions
- Add 20ul RNase A solution (20 mg/ml) before addition of Lysis Buffer FL 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 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 FEB provided with the kit.

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 FL

Suggestions
- Repeat the DNA purification procedure with a new sample. Vortex the mixture vigorously immediately after addition of Lysis Solution FL.

Possible cause
- Suboptimal elution of DNA from the column

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

Nucleo-pore® Stool DNA Mini 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 Nucleo-pore® Stool DNA Mini 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 Nucleo-pore® Stool DNA Mini 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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