



GENETIX BRAND

HANDBOOK

 **Nucleo-pore[®]**

Nucleo-pore[®] gDNA Fungal/Bacterial Mini Kit

Nucleo-pore[®] gDNA Fungal/Bacterial DNA Kit NP-7006D 50 Preps



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COMPONENTS

Kit contents

Nucleo-pore® gDNA Fungal/Bacterial Mini Kit

Cat. No.	NP-7006D
No of preps	50
Lysis Buffer FBL	40ml
DNA Binding Buffer* FBB	100ml
Pre-Wash Buffer* FBPW	15ml
Wash Buffer FBW	50ml
Elution Buffer FBE	10ml
ThrashingBead Tubes	50
FB Shredder Columns	50
FB Spin Columns	50
Collection Tubes	150
Handbook	1

* Please see "Preparation of Reagents"

Reagents, consumables and equipments not provided with the kit

- Ethanol (96-100%)
- 1.5 ml Micro centrifuge tubes
- Pipettes, Vortex, Dry/Waterbath

SAFETY INSTRUCTIONS

DNA Binding Buffer FBB

Contains Guanidine Thiocyanate (Component A): R 20/21/22, R 32; S 22, S 24-25, S45.
Contain Beta-Mercaptoethanol (Component B): R25, R27, R34, R51; S36/37/39

Pre-Wash Buffer FBPW

Contain Isopropanol: R11, R36, R67; S7, S16, S24/25, S26

Wash Buffer FBW

Contain Ethanol (Component A): R11, S7, S16
Contain Isopropanol (Component B): R11, R36, R67; S7, S16, S24/25, S26

Risk and Safety Phrases

R11- Highly flammable, R 20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
R25: Toxic if swallowed, R27: Very toxic in contact with skin, R 32 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, S 22 Do not inhale, S 24-25 Avoid contact with skin and eye, S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, S36/37/39: Wear suitable protective clothing, gloves and eye/face protection, S 45 In case of accident or if you feel unwell, seek medical advice immediately.

INTRODUCTION

Principle and Procedure

The Nucleo-pore gDNA Fungal/Bacterial Mini Kit utilizes 15 minute protocol for the isolation of DNA from tough-to-lyse fungi, mycelium and Gram (+) and (-) bacteria. Samples homogenized using Thrashing Beads followed by Lysis without using organic denaturants or proteinases. After two washing steps pure DNA is isolated using FB Spin column technology. Purified DNA is ideal for downstream molecular-based applications.

Specifications of gDNA Fungal/Bacterial Mini Kit

The gDNA Fungal/Bacterial Mini Kit is designed for the simple, rapid isolation of DNA (upto 25ug) from tough-to-lyse fungi, including *A. fumigatus*, *C. albicans*, *N. crassa*, *S. cerevisiae*, *S. pombe*, as well as from mycelium and Gram (+) and (-) bacteria. The procedure is easy and can be completed in as little as 15 minutes: DNA from Upto 50-100 mg (wet weight) fungi or bacteria or up to 200 mg tissue, or approximately 10⁹ bacterial cells, 10⁸ yeast cells and 10⁷ mammalian cells can be extracted using Fungal Bacterial DNA Kit. Kit also purifies high quality DNA ($A_{260}/A_{280} > 1.8$) from various sample sources like spores, pollen, nematodes, as well as other microorganisms.

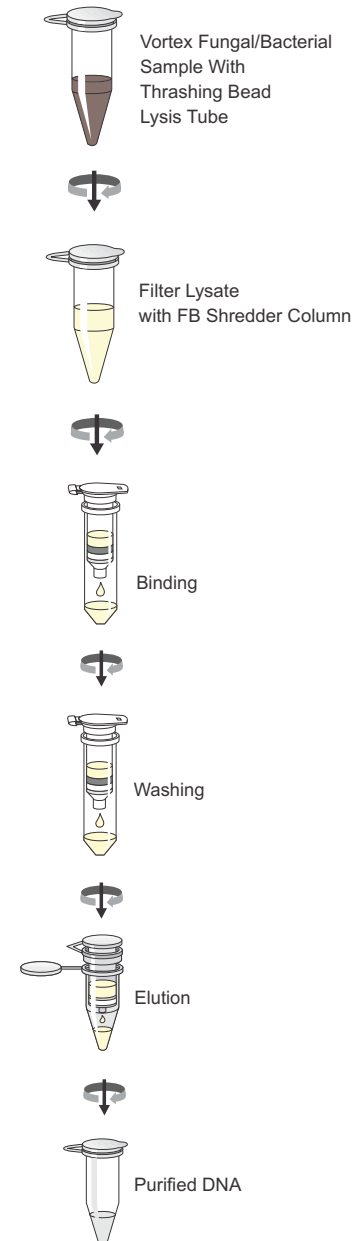
Preparation and Storage of Reagents

DNA Binding Buffer*FBB

Add 500 μ l beta-mercaptoethanol to 100 ml (0.5%(v/v) of DNA binding buffer

All kit components can be stored at room temperature for a period of one year from date of purchase.

DNA Purification from Fungus/Bacteria



Protocol for Isolation of Fungal/Bacterial DNA

Things to do before starting

- Check whether beta mercaptoethanol has been added to **DNA Binding Buffer** as per instructions given in "Preparation of Reagents"

Procedure

1. **Take 50-100 mg (wet weight) fungal or bacterial cells resuspended in up to 200 µl of water or 1X phosphate buffer saline or up to 200 mg of tissue to a ThrashingBead Tube. To it add 750 µl Lysis Buffer FBL.**
2. **Put the tube in a bead beater and process at maximum speed for 5 minutes, or vortex the ThrashingBead Tube for 5minutes**

Note: See manufacturer's literature for operating information of respective bead beater used.
3. **Centrifuge the Thrashing Bead Tube in a microcentrifuge at 10,000 x g for 1 minute.**
4. **Transfer up to 400 µl supernatant to a FB Shredder Column placed in a Collection Tube and centrifuge at 7,000 rpm (~7,000 x g) for 1 minute.** Snap off the base of the Shredder Column prior to use.
5. **Add 1,200 µl of DNA Binding Buffer FBB to the filtrate in the Collection Tube from Step 4.**
6. **Transfer 800 µl of the mixture from Step 5 to a FB Spin Column contained in collection tube and centrifuge at 10,000 x g for 1 minute.**
7. **Discard the flow through from the Collection Tube and repeat Step 6.**
8. **Add 200 µl Pre-Wash Buffer FBPW to the FB Spin Column in a new Collection Tube and centrifuge at 10,000 x g for 1 minute.**
9. **Add 500 µl Wash Buffer FBW to the FB Spin Column and centrifuge at 10,000 x g for 1 minute.**
10. **Put the FB Spin Column to a fresh 1.5 ml microcentrifuge tube and add 100 µl (minimum 25µl) Elution Buffer FEB in the center of column matrix without wetting the rim. Allow it to stand for 1 minute at room temperature. Centrifuge at 10,000 x g for 1 minute to elute pure DNA.**

TROUBLESHOOTING GUIDE

Poor DNA Yield

Possible cause

- Incomplete Lysis

Suggestions

- Sample not thoroughly homogenized. The mixture has to be vortexed vigorously (step 2). Elution efficiencies decrease dramatically, if elution is done with buffers with a pH < 7.0. Use slightly alkaline elution buffers like Buffer FEB (pH 8.5).
Check whether beta mercaptoethanol was added to the Binding Buffer FBB.

Clogged Columns

Possible Cause

- Too much sample material used, or sample not thoroughly homogenized.

Suggestions

- Do not take excess of sample. Vortex vigorously (Step 2).

Suboptimal Performance of DNA in enzymatic reactions

Possible Cause

- Contamination of DNA with inhibitory substances

Suggestions

- Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer

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 Kit	50	NP-7006D

Product Warranty

Nucleo-pore® gDNA Fungal/Bacterial 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® gDNA Fungal/Bacterial 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® gDNA Fungal/Bacterial 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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