



GENETIX BRAND

HANDBOOK

 **Nucleo-pore[®]**

Nucleo-pore[®] Yeastlytic Fungal DNA Extraction Kit

Purification of Fungal DNA

NP-2002D

40 Preps



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COMPONENTS

Kit contents

Nucleopore® Yeastlytic Fungal DNA extraction kitt

Cat. No.	NP-2002D
Number of Preps	40 preps
YL Enzyme (lyophilized , supplied with storage buffer)	1000 Units
Digestion Buffer	4.8 ml
Lysis Buffer	4.8 ml
Wash Buffer Concentrate	6 ml
Spin Columns	40
2ml Collection Tubes	40
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*Please see "Preparation and Storage of reagents"

STORAGE

Nucleopore® Yeastlytic Fungal DNA extraction kit should be stored dry at room temperature (18–26°C) except YL Enzyme, which is kept at -20°C after reconstitution. Kits can be stored for up to 12 months without showing any reduction in performance and quality.

Reagents, consumables, and equipment not supplied with the kit

- Ethanol
- Chloroform
- Microcentrifuge

SAFETY INSTRUCTIONS

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).

YL Enzyme Storage Buffer

Contains Beta-Mercaptoethanol: Highly toxic by skin absorption ,toxic by inhalation, irritant.
Risk and Safety phrases: R25/27/34/51, S36/37/39.

INTRODUCTION

Principle and Procedure

Nucleopore® Yeastlytic Fungal DNA extraction kit is designed for reliable and efficient isolation of genomic DNA from a broad spectrum of fungus species, including *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus nivosus* var *aureus*, *Candida albicans*, *Pichia pastoris*, and any fungi whose cell walls are susceptible to yeast lytic enzyme lysis. The kit is based on highly efficient enzyme lysis and fast spin column technology. Each standard prep yields about 7-20 μg of DNA with a size distribution of 35-60kb. The resulting genomic DNA can be used directly for all molecular biology analysis such as Southern Blot, PCR, restriction enzyme digestion, etc

Specifications of Nucleopore® Yeastlytic Fungal DNA extraction kit

- DNA Purity – High-quality, purified DNA
- DNA Size Limits ranges from ~35 bp to 60kb.
- Typical DNA recovery is 7- 20 μg total DNA per column
- Efficient DNA isolation from a broad spectrum of fungus species:
 - Aspergillus fumigatus
 - Aspergillus nidulans
 - Aspergillus nivosus var. aureus
 - Candida albicans
 - Pichia pastoris
 - Saccharomyces cerevisiae
 - Schizosaccharomyces pombe

Preparation and Storage of reagents

Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml Wash Buffer concentrate.

Add 200 μl of the supplied Storage Buffer to the lyophilized YL Enzyme Mix to dissolve the enzyme completely, spin briefly in a micro-centrifuge. Store the reconstituted YL Enzyme at -20°C.

Protocols

Protocol for Purification of Fungal DNA

Things to do Before starting:

- Check if wash buffer is prepared as per instructions
- Protocol I and II are almost same, except that chloroform is used in Protocol I. Protocol I usually gives more recovery of DNA by 30-80% compared to Protocol II. Protocol II is chloroform-free.

Protocol I

The kit works with either fresh cells or aged cells in either plates or liquid cultures. The following procedure is based on 1-1.5 ml culture ($1-5 \times 10^7$ cells). Increasing the amount of cells above the recommended level may cause overloading of the system.

1. Spin 1-1.5 ml of cells down briefly or centrifuge at 4000 rpm for 2 minutes. Remove the supernatant completely.
2. Add 120 μl of Digestion Buffer and 5 μl of YL Enzyme. Resuspend the pellet by vortexing and incubate at 37°C for 40-60 minutes
3. Add 120 μl of Lysis Buffer. Mix well by gently vortexing.
Note: vortex hard for 10-20 seconds after adding Lysis Buffer. This will increase your DNA recovery, but may result in shorter genomic DNA ranging from 20-35 kb. However, most of the DNA will remain more than 35 kb.
4. Add 250 μl of chloroform. Mix thoroughly for 1 minute.
5. Centrifuge in a table top centrifuge at > 10,000 rpm for 2 minutes.
6. Load the supernatant onto the Spin column and centrifuge at > 10,000 rpm for 1 minute.
7. Add 300 μl of Wash Buffer concentrate and centrifuge for 1 minute at $\geq 10,000$ rpm to wash.
8. Add another 300 μl of Wash Buffer concentrate to repeat the wash and centrifuge for 1 minute.
9. Transfer the Spin column to a new 1.5 ml centrifuge tube and add 60 μl of water or TE directly onto the membrane. Wait for one minute then centrifuge for 10 seconds to elute the DNA.

Protocol II

1. Spin 1-1.5 ml of cells down briefly or centrifuge at 4000 rpm for 2 minutes. Remove the supernatant completely.
2. Add 120 μ l of Digestion Buffer and 5 μ l of YL Enzyme. Resuspend the pellet by vortexing and incubate at 37°C for 40-60 minutes
3. Add 120 μ l of Lysis Buffer. Mix well by gently vortexing.
Note: vortex hard for 10-20 seconds after adding Lysis Buffer. This will increase your DNA recovery, but may result in shorter genomic DNA ranging from 20-35 kb. However, most of the DNA will remain more than 35 kb.
4. Centrifuge in a table top centrifuge at > 10,000 rpm for 2 minutes.
5. Load the supernatant onto the Spin column and centrifuge at > 10,000 rpm for 1 minute.
6. Add 300 μ l of Wash Buffer concentrate and centrifuge for 1 minute at \geq 10,000 rpm to wash.
7. Add another 300 μ l of Wash Buffer concentrate to repeat the wash and centrifuge for 1 minute.
8. Transfer the Spin column to a new 1.5 ml centrifuge tube and add 60 μ l of water or TE directly onto the membrane. Wait for one minute then centrifuge for 10 seconds to elute the DNA.

TROUBLESHOOTING GUIDE

Low Yield

Possible cause:

- The initial amount of yeast cells used is inappropriate

Suggestion(s)

- The cultures of certain fungi strains can reach very high density. In this case using less volume of cells, such as using 0.4-0.8 ml instead of using 1-1.5 ml as suggested. Also, too many cells can easily overload the system. Try to use less cells when you suspect that cell lysis is incomplete. You should be able to see that cells are lysed after the incubation with the enzyme in step 2 of both Protocol I and II
- Fresh and early log phase cells are usually more susceptible to yeast lytic enzyme lysis than aged cells. Try to use fresh cultures whenever possible.
- Susceptibility to yeast lytic enzymes varies for different yeast species. If you see incomplete lysis, extend the first incubation time up to 2 hours or over 16 hours.

Low A260/A230 ratio

Possible cause:

- Column tip contaminated

Suggestion

- When removing the column from the collection tube, be careful that the tip of the column does not come into contact with the flowthrough. Trace amounts of salt from the flowthrough can contaminate a sample resulting in a low A260/A230 ratio. Ethanol contamination from the flowthrough can also interfere with DNA elution. Spin columns are designed for complete elution with no buffer retention or carryover.

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® Yeastlytic Fungal DNA Extraction 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® Yeastlytic Fungal DNA Extraction 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® Yeastlytic Fungal DNA Extraction 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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