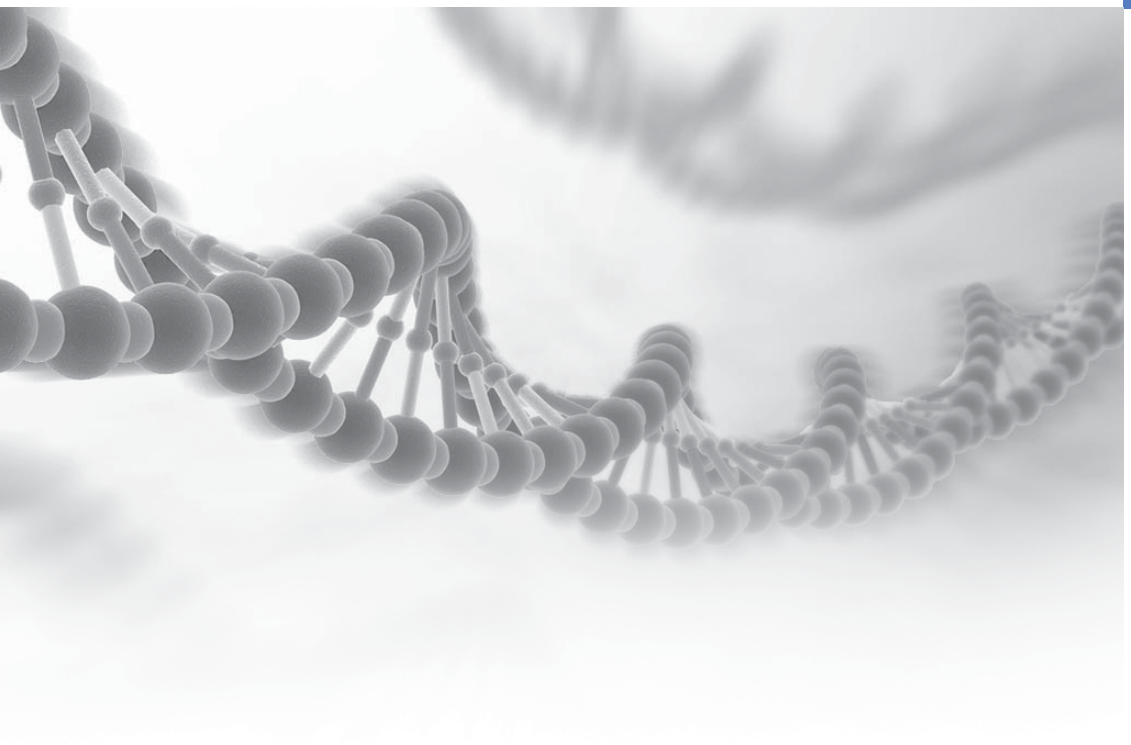
GENETIX BRAND

HANDBOOK

Nucleo-pore®

Nucleopore® Yeast Transformation Kit



☐ **Nucleopore® Yeast Transformation Kit** **NP- 1002T** **120 Preps**

genetix Biotech Asia Pvt. Ltd.

Genetix Biotech Asia Pvt. Ltd.
71/1, First Floor, Shivaji Marg, Najafgarh Road, New Delhi - 110015
Phone : +91-11-45027000 ■ Fax : +91-11-25419631
E-mail : info@genetixbiotech.com ■ www.genetixbiotech.com

www.genetixbiotech.com

Nucleo-pore®

Contents

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COMPONENTS

Kit contents

Nucleopore® Yeast Transformation Kit

Cat #	NP-1002T (120 preps.)
YT 1 Buffer	60ml
YT 2 Buffer	6ml
YT 3 Buffer	60ml
Handbook	1

Reagents, consumables and equipments not provided with the kit

- 1.5 ml microcentrifuge tubes
- Disposable pipette tips
- Manual pipettes
- Centrifuge
- Vortex

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).

INTRODUCTION

Principle and Procedure

The Nucleopore® Yeast Transformation kit makes yeast transformations easier and more efficient than protocols that are currently in use. It improves the efficiency and reliability of its predecessor. The competent yeast cells prepared with these reagents can be used immediately for transformation experiments or can be stored frozen at or below -70°C for direct use in the future. This method is suitable for both circular and linear plasmid transformations.

Kits is designed for 120 regular or 600 micro-scale transformation experiments.

Specifications of Nucleopore® Yeast Transformation Kit

- Fast and easy steps to make competent yeast cells within 10 minutes.
- Easy, single step transformation procedure that takes less than an hour.
- Simple method for multiple plasmid transformations.
- High transformation efficiency that yields approximately 10^5-10^6 transformants per μg plasmid DNA (circular).
- Broad spectrum (S. cerevisiae, C. albicans, S. pombe, Pichia pastoris).

Optimizing Condition for Higher Efficiency

The following factors need to be considered if the experiments require high transformation efficiency:

Cell growth state: Cells used should be in mid-log phase. Early or late log-phase cells yield relatively fewer transformants.

Cell density: Optimal cell density should be between 5×10^6 and 2×10^7 cells/ml (0.8-1.0 OD600), but in most cases use of cultures with cell densities at the high end of the range greatly increases transformation efficiency in comparison to cultures with cell densities at the low end of the range. The optimal cell density can also be achieved by resuspending the cells in a smaller volume of YT 2 Buffer

Incubation time after adding YT 3 Buffer: An incubation time of 45 minutes is good for general purposes, transformation efficiency is much better with longer incubation times (up to 2-3 hours in most cases). Again, results vary according to strain used.

Amount of DNA used: For transformation experiments using circular DNA such as 2-based plasmids, the efficiency stops increasing linearly as you increase the DNA above 1 μg using the standard transformation protocol. For integrative transformation, purity and amount of DNA used are important. Higher amount of linearized DNA is recommended to achieve the best results (up to 5 μg of DNA can be used in the standard transformation protocol).

Pre-warm solutions: Usually, it is not necessary to pre-warm all the solutions. For most purposes, cold solutions from the refrigerator will work fine. But, if your experiment requires maximum transformation efficiency, such as in library screening, the pre-warming of the buffer before the transformation experiment will result in higher efficiency. Pre-warm these amounts to 20-37°C before doing experiments.

Outgrowth after transformation: Some selection markers and certain yeast strains need outgrowth step in rich medium for high efficiency transformation. Add 4 volumes of YPD medium to the transformation mixture at the end of transformation, (2 ml YPD for the Standard Transformation) mix, incubate at 30°C for 2 hours. Spin the cells down, resuspend the cells in 2 ml of water, spin the cells down again to wash the cells, resuspend the cells in original (0.5 ml for Standard Transformation Protocol) or less volume of water for plating on selection plates.

Preparation & Storage of Reagents

The kit is designed for 120 regular or 600 micro-scale transformation experiments and is stable for 1 year at 4°C.

PROTOCOL FOR YEAST TRANSFORMATION

Standard Protocol for Yeast Transformation

Things to do before starting

- Check if Buffers were Pre-warmed at 20-37°C before doing experiments.
- Please read Optimizing Conditions for Higher Efficiency.

Procedure

For maximum transformation efficiency, please read "Optimizing Your Conditions for Higher Efficiency,". If your experiment requires larger scale than the "Standard Transformation protocol" you can increase the volume in each step proportionally according to this procedure.

Preparation of Competent Cells

Grow yeast cells at 30°C in 10 ml YPD broth until mid-log phase is achieved (~5×10^6 - 2×10^7 cells/ml or OD600 of 0.8-1.0).

a. Pellet the cells at 500 x g for 4 minutes and discard the supernatant.

b. Add 10 ml YT 1 buffer to wash the pellet.

c. Repellet the cells and discard the supernatant.

d. Add 1 ml YT 2 buffer to resuspend the pellet.

At this point, the competent cells can be used for transformations directly or stored frozen at or below -70°C for future use. It is important to freeze the cells slowly. To accomplish this, either wrap the aliquotted cells in 2-6 layers of paper towels or place in a Styrofoam box before placing in the freezer. DO NOT use liquid nitrogen to snap-freeze the cells.

Transformation

The procedure is the same for both frozen stored (thawed at room temperature) and freshly prepared competent yeast cells.

1. Mix 50 µl of competent cells with 0.2-1 µg DNA (in less than 5 µl volume); add 500 µl YT 3 buffer and mix thoroughly.
2. Incubate at 30°C for 45 minutes. Mix vigorously by vortexing 2-3 times during this incubation.
3. Spread 50-150 µl of the above transformation mixture on an appropriate plate. It is unnecessary to pellet and wash the cells before spreading.
4. Incubate the plates at 30°C for 2-4 days to allow for growth of transformants.

Note: For transformations of C. albicans, use freshly prepared competent cells; frozen cells sometimes give poor results.

SUPPLEMENTARY PROTOCOL FOR MULTIPLE-PLASMID TRANSFORMATIONS

Things to do before starting

- Check if Buffers were Pre-warmed at 20-37°C before doing experiments.
- Please read Optimizing Conditions for Higher Efficiency.

Procedure

This protocol can generate enough transformants for most applications. If your work requires a large number of transformants as in library screening, use "Standard Protocol for Yeast Transformation"

Preparation of Competent Cells: same as "Standard Protocol for Yeast Transformation " .

Transformation: This can be done either in 96-well plates or tubes.

1. Add 10 µl competent cells to DNA (0.2-1 µg), mix by tapping or lightly vortexing; add 100 µl YT 3 buffer and mix thoroughly.
2. Incubate at 30°C for 60-90 minutes. Mix vigorously 2-3 times during this incubation.
3. Directly spread the transformation mixture onto 1-2 plates.
4. Incubate the plates at 30°C for 2-4 days to allow for growth of transformants.

TROUBLESHOOTING GUIDE

Suboptimal yeast competent cells

Possible cause
- Less no of cells in starting culture

Suggestions
- Make sure that the culture was in log-phase growth at the time the cells were harvested for making competent cells. If the overnight culture grew slower than expected (or not at all), start over by preparing a fresh overnight culture. Failure to thoroughly disperse the colony used for the inoculum will result in slow growth.

Possible cause
- No cells in starting culture

Suggestions
- Obtaining a healthy liquid culture, streak a fresh working stock plate (from the frozen glycerol stock) and inoculate with a fresh colony.

Possible cause
- Buffers were not Pre-warmed

Suggestions
- For most purposes, cold solutions from the refrigerator will work fine. But, if your experiment requires maximum transformation efficiency, pre-warming of the buffer before the transformation experiment will result in higher efficiency. Pre-warm Buffer to 20-37°C before doing experiments

Plate is confluent with growth

Possible cause
- Contamination of culture with bacteria or other microbe

Suggestions
- Streak out your stock yeast culture to ensure no contamination is present. Check liquid medium for contamination

Possible cause
- Yeast cells were plated onto wrong medium

Suggestions
- Check the genotype of your strain, plasmid marker and medium used

Possible cause
- Wrong dilution was plated

Suggestions
- Always plate a range of dilutions to ensure isolation of individual transformants

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® Yeast Transformation 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® Yeast Transformation 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® Yeast Transformation 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.

Please contact:
Genetix Biotech Asia (P) Ltd.
71/1, Najafgarh Road, Shivaji Marg,
New Delhi. 110015.
INDIA.
E-mail: info@genetixbiotech.com
techsupport@genetixbiotech.com

Tel: +91-11-45027000
Fax: +91-11-25419631