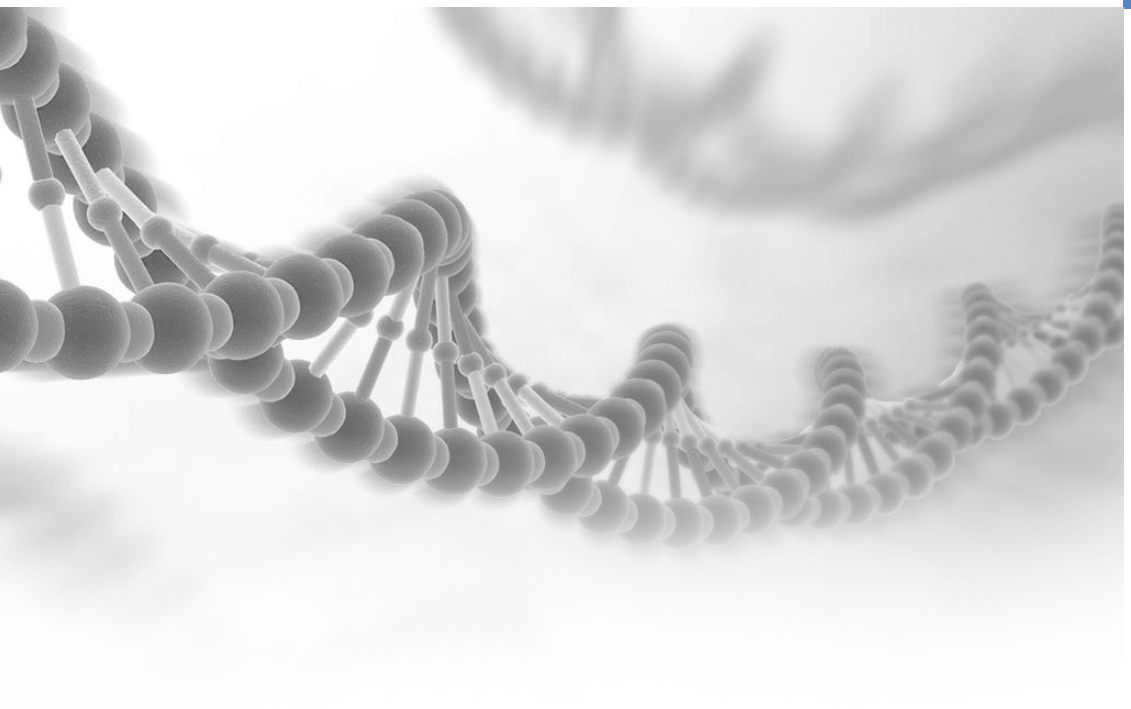
GENETIX BRAND

HANDBOOK

Nucleo-pore®

Nucleo-pore® *E. coli* Transformation Kit



☐ **Nucleo-pore® *E. coli* Transformation Kit** **NP- 1003T**

For 20ml Competent Cell preparation

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Nucleo-pore®

Contents

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COMPONENTS

Kit contents

Nucleo-pore® *E. coli* Transformation Kit

Cat #	NP-1003T (for 20ml Competent cell preparation)
Broth TB	200 ml
Wash Buffer(2x) TWB *	10ml
Competent Buffer(2x) TCB*	10ml
Dilution Buffer TDB	20ml
Instruction Manual	1

*Please see "Preparation and Storage of reagents"

Reagents, consumables and equipments not provided with the kit

- 1.5 ml Micro centrifuge tubes
- 15 ml and 50 ml centrifuge tubes
- Pipettes, Vortex, Dry/Waterbath
- Petri plates, spreader

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.

Competent Buffer TCB (2X Stock)
Risk phrases: R 36/38
Safety phrases: S 26, S36

R 36/38 :Irritating to eyes and skin, S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, S 36: Wear suitable protective clothing.

INTRODUCTION

Principle and Procedure

The E. coli Transformation Kit is a simple 3 steps protocol; culturing the desired E. coli strain in Broth TB medium, washing and resuspending the cells in the specially formulated buffer sets for highly efficient DNA transformation.

The protocol eliminates the requirement of heat-shocking and related procedures. The kit provides a special supplementary protocol for directly adding DNA to prepared Competent cells and the mixture spread on to a culture plate. Transformation efficiencies typically range from 10^8–10^9 transformants/µg of pUC19 DNA, but can vary depending on the strain of E. coli.

Specifications of Nucleo-pore *E. coli* Transformation Kit

Fast and rapid method for generation of reliable & highly efficient competent cells for DNA Transformation. The Competent cells can be transformed in seconds with special fast protocol, without heat shocking, lengthy incubations, or outgrowth steps.

A typical Transformation will have $>10^8$ transformants/µg plasmid DNA. The Kit features a specially formulated Broth TB as a growth medium which increases E. coli transformation efficiency, to a typically of 5 to 100-fold for most lab strains.

Preparation and Storage of reagents

Wash Buffer TWB

Prepare 5 ml of 1X Wash Buffer by adding 2.5 ml Dilution Buffer to 2.5 ml of Wash Buffer (2X Stock) .

1X buffer should be stored on ice prior to use. The 1X buffer are stable for about 2 days when stored at 0 - 25°C.

Competent Buffer TCB

Prepare 5 ml of 1X Competent Buffer by adding 2.5 ml Dilution Buffer to 2.5 ml of Competent Buffer (2X Stock). 1X buffer should be stored on ice prior to use. The 1X buffers are stable for about 2 days when stored at 0 - 25°C.

The kit components are guaranteed for up to one year from date of purchase when kept at mentioned temperatures.

TRANSFORMATION EFFICIENCY

E. coli Strains

Different E. coli strains vary in their ability to be transformed with DNA. Strains like JM109, C600, TG1, DH5α*, XL10 Gold*, and BL21 and its derivatives typically yield the best results when prepared with the E. coli Transformation Kit.

Incubation Time

The Supplementary protocol can be used for most transformations using Ampicillin selection and not requiring outgrowth. The highest transformation efficiencies can be obtained by incubating Competent cells with DNA on ice for 2-5 minutes prior to plating.

Prewarming Culture Plates

Chilled plates will decrease Competent cell transformation efficiency. It is recommended that culture plates be pre-warmed to >20°C (preferably 37°C) prior to plating.

Addition of SOC Medium to Transformation Mixtures (Outgrowth)

When selecting with Kanamycin, Tetracycline, etc., an outgrowth performed in SOC medium is required for efficient transformation. In most cases, this step can be omitted when selecting with Ampicillin. After the transformation mixture has incubated on ice for 5-10 min, add 4 volumes of SOC (400 µl of SOC to 100 µl of transformation mixture) and incubate for 1 hour at 37°C with gentle shaking at 200-300 rpm. Afterwards, spread the mixture directly onto pre-warmed culture plates. Reducing agents [e.g., DTT (Dithiothreitol) and 2-ME (β-mercaptoethanol)] are not required for this procedure.

Culture Conditions

E. coli cells become highly competent when cultured at 20-33°C prior to preparation. Higher temperatures (i.e., 33°C-37°C) can decrease the transformation efficiency 2 to 10-fold. Also, cells can be harvested at lower densities (e.g., OD_{600nm} 0.2-0.4) and resuspended in smaller volumes (e.g., 1-3 ml vs. 5 ml as recommended in the standard procedure). Cells harvested at lower densities (OD_{600nm} 0.2-0.6) are usually "more competent" than those cells harvested at higher densities (OD_{600nm} >0.6).

Protocol for Nucleo-pore E. Coli Transformation Kit

Things to do before starting

- Check whether Wash Buffer TWB was prepared as per instructions.
- Check whether Competent Buffer TCB was prepared as per instructions
- It is important that Steps 2-5 be performed at 0-4°C.
- Refer to the instructions given in "Preparation & storage of reagent" section

Procedure

1. Take 50 ml of fresh, overnight E. coli culture grown in LB to inoculate 50 ml Broth TB in a 500 ml culture flask. Shake culture vigorously (150 - 250 rpm) at the appropriate temperature until the OD600nm is 0.4 - 0.6.

2. Transfer the overnight grown culture from Step 1 to ice. After 10 minutes, pellet the cells by centrifugation at 3,000 - 3,700 rpm (i.e., 1,600 - 2,500 x g) for 10 minutes at 0 - 4°C.

3. Discard the supernatant and resuspend the cells gently in 5 ml ice-cold 1X Wash Buffer TWB. Re-pellet the cells as in Step 2.

4. Discard the supernatant formed completely and gently resuspend the cells in 5 ml ice-cold 1X Competent Buffer TCB.

5. Make different aliquot of 0.1-0.2 ml of the cell suspension into sterile microcentrifuge tubes on ice.

   Competent Cells are now ready for transformation with DNA or can be stored at -70°C for transformation at a later time.

Supplementary protocol for fast E. coli Transformation

The fast transformation procedure can be used for most transformations using Ampicillin selection and not requiring outgrowth. The highest transformation efficiencies are obtained by incubating competent cells with DNA on ice for 2-5 minutes prior to plating.

Things to do before starting

- All Steps should be performed at 0-4°C.
- Prewarm culture Plates to >20°C (preferably 37°C) prior to plating

Supplementary Protocol

1. Add 1-5 µl plasmid DNA to a tube of thawed Competent cells on ice, mix gently for a few seconds

   Note: For best results try to keep the added volume of DNA less than 5% of the total.

2. Spread 50-100 µl of the mixture onto a pre-warmed (37°C) culture plate containing Ampicillin.
3. Incubate the plate at the 37°C temperature for the colonies to grow.

Note: For Ampicillin selection only.

APPENDIX

Although SOB has traditionally been used for Competent cell preparation, Broth TB is now the medium of choice for the generation of Competent E. coli that exhibit fast and highly efficient transformation kinetics. However, SOB can still be used in both Competent E. coli Transformation Kit and Buffer Set procedures.

SOB Recipe: (1 Liter)

Mix the following ingredients:

20 g Bacto-tryptone 0.58 g NaCl (or 2 ml of 5M NaCl) 10 ml 1M MgCl2

5 g Yeast extract 0.19 g KCl (or 0.5 ml 1M KCl) 10 ml 1M MgSO4

Add ddH2O to a total volume of 1 liter.

Adjust pH to 6.0-7.0 with NaOH.

Autoclave at 10 psi for 15-20 minutes.

SOC Recipe: (100 ml)

Add 1 ml of a 2 M filter-sterilized glucose solution or 2 ml of 20% (w/v) glucose solution to 100 ml of SOB medium.

TROUBLESHOOTING GUIDE

Low yield or no transformants

Possible cause
- Low transformation efficiency of competent E. coli cells.

Suggestions
- Check transformation efficiency with 0.1 ng of a supercoiled vector DNA, e.g. pUC19. The competent cells should yield at least $1x10^6$ transformants per µg of supercoiled DNA, which corresponds to 100 colonies, when 0.1 ng of plasmid had been used for transformation.

Possible cause
- Cloned sequence is not tolerated by E.coli.

Suggestions
- Check the target sequence for strong E.coli promoters or other potentially toxic elements, as well as inverted repeats. In cases where the product of a cloned gene is toxic to the host, use promoters with a very low expression background or choose a low copy plasmid as cloning vehicle.

Empty vector (no insert)

Possible cause
- Vector recircularization

Suggestions
- Dephosphorylate the vector prior to use. Vector dephosphorylation is recommended in all cases, including cloning strategies where the vector ends are incompatible for recircularization. Ensure the phosphatase is completely inactivated or removed after dephosphorylation.

Possible cause
- Incomplete cleavage of the vector.

Suggestions
- Check the cleavage efficiency on an agarose gel. If it is difficult to achieve complete cleavage, gel-purify the linear form of the vector .

Incorrect Constructs

Possible cause
- Non-specific PCR product cloned.

Suggestions
- Gel-analyze the PCR product prior to ligation. If non-specific PCR products or primer-dimers were generated during the PCR reaction, gel-purify the target PCR product. Smaller DNA fragments present in the PCR mixture are ligated more efficiently with the cloning vector and outcompete the target PCR products.

Possible cause
- Truncated insert due to contaminating endo- or exonucleases.

Suggestions
- Use only the highest quality enzymes for cloning, e.g. LO-tested enzymes and exclude any possibility of endo-, exonuclease and phosphatase contamination in enzyme preparations..

Colonies without plasmid

Possible cause
- Insufficient amount of antibiotic in agar medium.

Suggestions
- Use 100 µg/ml of ampicillin in LB-ampicillin agar plates. Allow the LB medium to cool to 55°C before adding the antibiotic. Ampicillin is sensitive to light - long-term exposure to light can lead to low ampicillin concentration in plates.

Possible cause
- Satellite colonies.

Suggestions
- Some fast growing E.coli strains degrade ampicillin faster, which leads to formation of smaller satellite colonies around transformants after >16 hours of incubation. Use shorter incubation times and do not use small satellite colonies for clone analysis.

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® *E. coli* 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 Nucleo-pore® *E. coli* 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 Nucleo-pore® *E. coli* 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.

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