



GENETIX BRAND

HANDBOOK

 **Nucleo-pore[®]**

Nucleopore Genomic DNA Clean & Concentrator Kit 25 Prep

Nucleopore Genomic DNA Clean & Concentrator Kit NP-0104D



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COMPONENTS

Kit contents

Nucleopore Genomic DNA Clean & Concentrator Kit

Cat #	NP-0104D
No. of Preps	(25 preps.)
Binding Buffer (DIP)	50 ml
Wash Buffer Concentrate (DWB)	1.6 ml
Elution Buffer (DEB)	1 ml
gELSpin Columns	25
Collection Tubes	50
Handbook	1

*Please see "Preparation and Storage of reagents"

STORAGE

Nucleopore Genomic DNA Clean & Concentrator kits should be stored dry at room temperature (18–26°C). Kits can be stored for up to 12 months without showing any reduction in performance and quality.

Reagents, consumables and equipments not provided with the kit

- Ethanol
- 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 (MSDSs).

Binding Buffer DIP

Contain Guanidine Hydrochloride: R&S Phrases: R10-22-36/38, S7-16

R10: Flammable, R22 Harmful by Inhalation, in contact with skin and if swallowed, R36/38: Irritating to eyes and skin. S7: Keep container tightly closed. S16: Keep away from sources of ignition- No Smoking.

Follow the safety guidelines and rules enacted by your research institution or facility .

INTRODUCTION

Principle and Procedure

Nucleopore Genomic DNA Clean & Concentrator Kit is for the quick (5 minute) recovery of ultra-pure, large-sized DNA (e.g., genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, DNA, etc.) from any enzymatic reaction or impure preparation (e.g., Proteinase K digestion). There is no need for organic denaturants, chloroform, or messy precipitations: simply add the specially formulated Binding Buffer DIP to a sample and then transfer the mixture to the gEL-Spin Column. Eluted DNA is suitable for sequencing, PCR, endonuclease digestion, and other enzymatic procedures. The product is also compatible with smaller DNAs (50 bp to 10 kb) from PCR, digestions, crude plasmid preparations, cDNA synthesis,

Specifications of Nucleopore Genomic DNA Clean & Concentrator Kit

- Quick (5 minute) method for cleaning and concentrating Genomic DNA.
- Ideal for high-quality (A_{260/280} ≥ 1.8) high molecular weight DNA for ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.
- Capable of purifying small DNA fragments >50 bp and large sized DNAs >200 kb.
- Up to 10 µg total DNA per column can be eluted into as ≥ 10 µl of low salt DNA Elution Buffer or water. Recovery of DNA ranges from 70-95%.
- Sample Sources – DNA from impure preparations of genomic DNA (e.g., Proteinase K digestions), plasmid DNA (including BAC), viral DNA, and whole genome amplified (wga) DNA. Can also be used for the purification of low molecular weight DNA (50 bp to 10 kb) from PCR, endonuclease digestion, post-RT cDNA synthesis, etc..
- Product Detergent Tolerance – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 1% SDS.

Preparation and Storage of reagents

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

PROTOCOL

Things to do before starting

- All centrifugation steps should be performed between 10,000 - 16,000 x g.
- Check if wash buffer is prepared as per instructions

Protocol for Purification and Concentration of Genomic DNA

1. In a 1.5 ml microcentrifuge tube, add 2-5 volumes of Binding Buffer DIP to each volume of DNA sample (see table below). Mix thoroughly.

Application	Binding Buffer : Sample	Example
Plasmid, genomic DNA (>2 kb)	2 : 1	200 µl : 100 µl
PCR product, DNA fragment	5 : 1	500 µl : 100 µl

Note: It may be necessary to add RNase A to cell lysate prior to load and spin a column multiple times if a sample has a volume larger than 1ml.

2. Transfer mixture to a provided gEL Spin Column in a Collection Tube. Note: the maximum capacity of the gEL Spin column is 1ml, repeat the step if the lysate is more than 1ml.
3. Centrifuge for 30 seconds at 11,000 x g. Discard the flow-through.
4. Add 200 µl Wash Buffer DWB to the column. Centrifuge for 1 minute at 11,000 x g. Repeat the wash step.
5. Add ≥ 10 µl Elution Buffer DEB or water directly to the column matrix and incubate at room temperature for one minute. Transfer the column to a 1.5 ml microcentrifuge tube and centrifuge at for 30 seconds at 11,000 x g to elute the DNA. Ultra-pure DNA is now ready for use.

Note: To increase the yield, preheat the Elution Buffer DEB at 60-70°C prior to use.

TROUBLESHOOTING GUIDE

Low yield or no transformants

Possible cause

- Improperly Prepared/Stored DNA Wash Buffer

Suggestions

- Make sure ethanol has been added to the Wash Buffer concentrate DWB. Cap the bottle tightly to prevent evaporation over time

Possible cause

- Addition of Elution Buffer DEB

Suggestions

- Add elution buffer directly to the column matrix and not to the walls of the column. Elution buffer DEB requires contact with the matrix for at least 1 minute for large DNA ≥ 10 kb recovery.

Possible cause

- Incomplete Elution

Suggestions

- DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) and incubate for several minutes prior to elution
- Sequential elutions may be performed for quantitatively higher recovery but lower final DNA concentration. This is recommended for DNA ≥ 10 kb.

Low A260/A230 Ratios

Possible cause

- Column Tip Contaminated

Suggestions

- 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 low A260/A230 ratios. Ethanol contamination from the flowthrough can also interfere with DNA elution. gELSpin columns are designed for complete elution with no buffer retention or carryover.

Following Clean-up , Multiple Bands Appear in an Agarose Gel

Possible cause

- Acidification of DNA Loading Dye

Suggestions

- Most loading dyes do not contain EDTA and will acidify ($\text{pH} \leq 4$) over time due to some microbial growth. This low pH is enough to cause DNA degradation. Therefore, if water is used to elute the DNA, 6X Loading Dye containing 1 mM EDTA is recommended

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® Genomic DNA Clean & Concentrator 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® Genomic DNA Clean & Concentrator 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® Genomic DNA Clean & Concentrator 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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