



GENETIX BRAND

HANDBOOK

 **Nucleo-pore**[®]

Nucleopore BAC DNA Miniprep Kit - NP 8404D

Nucleopore BAC DNA Miniprep Kit NP 8404D (25 Preps)



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

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Nucleopore BAC DNA Miniprep Kit

Cat #	NP-8404D
Nucleopore BAC DNA Miniprep Kit	25 preps
Buffer BCP1	10 ml
Buffer BCP 2	10 ml
Buffer BCP 3	12 ml
Wash Buffer BEW	6 ml
Wash Buffer BPW (concentrate)	6 ml
Elution Buffer BCE	1 ml
BC Columns	25
Collection Tubes CT	50
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Safety Instructions

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Buffer BCP2 contains NaOH and BCP3 Buffer contains chaotropic reagents. Please use proper safety precautions with these reagents.

Product Description

Nucleopore BAC DNA Miniprep Kit is for efficient isolation of BAC plasmid DNA or other large plasmids (e.g., PAC) from E. coli cell lysates using a procedure that is simple, rapid, user-friendly, and reliable compared to kits offered from other suppliers. It features a modified alkaline lysis protocol together with unique spin column matrix technology to yield high quality plasmid DNA in minutes. Nucleopore BAC DNA Miniprep Kit features color-coded reagents that allow easy visualization and assessment of complete bacterial cell lysis and buffer neutralization. BC columns are optimized for high yield endotoxin-free plasmid DNA recovery. BAC DNA purified using the BAC DNA Miniprep Kit is well suited for sequencing, PCR, endonuclease digestion, etc.

Specification

- DNA Purity: High-purity and endotoxin-free (<50 EU/ μ g) plasmid DNA in low salt buffer or water; typical A(260/280) \geq 1.8. Eluted DNA is suitable for sequencing, endonuclease digestion, PCR, and other reactions requiring highly purified DNA.
- Recovery Volume: \geq 10 μ l.
- Plasmid DNA Size: Up to 200 kb.
- Plasmid DNA yield: Up to 10 μ g per preparation, depending on the plasmid copy number, initial volume of E. coli culture processed, and culture growth conditions. The typical yield is 1 μ g per 3 ml of culture.
- Procedure: Can be conducted at room temperature, between 15-30°C.

Buffer Preparation

Add 24 ml 100% ethanol to the 6 ml Wash Buffer BPW concentrate (48 ml ethanol to the 12 ml Wash Buffer BPW concentrate).

Protocol

1. Spin down 0.5 – 5 ml of bacterial culture to a pellet at 5,000-6,000 x g for 5 minutes in an appropriate tube and centrifuge. Discard supernatant.
2. Add 200 μ l of **Buffer BCP1** to the tube and resuspend pellet completely (i.e., by vortexing or pipetting). Transfer to a 1.5 ml microcentrifuge tube.
3. Add 200 μ l of **Buffer BCP2** and mix by inverting the tube 4-6 times. Cells are completely lysed when the solution appears clear, purple, and viscous. Proceed to the next step within 3 minutes. Wait 1-3 minutes before proceeding to Step 4.
4. Add 400 μ l of **Buffer BCP3** and mix gently but thoroughly. **Do not vortex vigorously.** The sample will turn yellow when the neutralization is complete. Wait 1-2 minutes before proceeding to Step 5.
5. Centrifuge sample(s) in a microcentrifuge for 3 minutes.
6. Place a **BC** column into a **Collection Tube** and transfer the supernatant from Step 5 into the column. When transferring the supernatant avoid carrying any cellular debris to the column and be careful not to disturb the green pellet.
7. Centrifuge the **BC column/Collection Tube** assembly for 30 seconds.
8. Discard the flow-through in the Collection Tube, making sure the flow-through does not touch the bottom of the column. Return the BCcolumn to the **Collection Tube3**.
9. Add 200 μ l of **Buffer BEW** to the column and centrifuge for 15 seconds.
10. Add 400 μ l of **Wash Buffer BPW** to the column. Centrifuge for 30 seconds. Empty the **Collection Tube** and centrifuge for an additional minute.
11. Transfer the column into a clean 1.5 ml microcentrifuge tube and then add \geq 10 μ l of **Elution Buffer BCE** to the column. Centrifuge for 30 seconds to elute the DNA.

TROUBLESHOOTING GUIDE:

Problem	Possible Causes and Suggested Solutions
<p>Low DNA Yield</p> <p>Culture growth conditions</p>	<ul style="list-style-type: none"> Poor aeration of culture. The optimal culture volume to air volume ratio is 1:4 or less (20% culture, 80% air). For best aeration, use baffled culture flasks, a vented or gas-permeable seal on the culture vessel, and incubate with vigorous shaking. Other possible reasons may include: An overgrown/ undergrown or contaminated culture, or omission of antibiotics from the growth medium. Use a fresh culture for optimal performance. Grow the culture to an O.D.600 > 1.0.
<p>Procedural errors</p>	<ul style="list-style-type: none"> Incomplete lysis: after addition of Buffer BCP2 the solution should change from opaque red to clear purple, indicating complete lysis. Different E. coli strains often require different growth conditions and may vary in their susceptibility to alkaline lysis. Incomplete neutralization: cell debris will float to the surface after centrifugation and the pellet may appear “puffy”. Make sure the neutralization is complete prior to centrifugation. Invert the tube an additional 2 - 3 times after the sample turns yellow following the addition of Buffer BCP3.
<p>Plasmid Wash Buffer</p>	<ul style="list-style-type: none"> Ensure that ethanol has been added to the wash buffer concentrate.

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

SurePrep® Buffer Set**	1	15143-BS
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Plant Kit	50 preps	NP-84905
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Midi Kit	12 preps	NP-80043
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 BAC DNA Miniprep 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 BAC DNA Miniprep 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 BAC DNA Miniprep 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

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