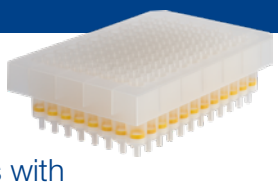


NucleoSpin® 96 Plasmid Transfection-grade Plus

Automated high throughput plasmid DNA purification using silica membrane-based kits with Eppendorf's epMotion® liquid handling workstations

Application benefits

Elevate your nucleic acid purification processes using MACHEREY-NAGEL's DNA/RNA purification kits in conjunction with Eppendorf's epMotion® liquid handling system:

- Flexible sample numbers: Process up to 96 samples in parallel
- Consistent recovery of plasmid DNA ensuring reliable reproducibility in both yield and purity
- Optimized endotoxin removal
- Optimize speed while minimizing plastic consumption
- For questions about reagents or script support please contact: support@mn-net.com

Keywords

Plasmid DNA, automated DNA purification, liquid handling system, high-purity, high-yield, transfection

Eppendorf epMotion® 5075vt
The Eppendorf epMotion® 5075vt offers versatility through the incorporation of various pipetting modules, a gripper, heater shaker, and a vacuum chamber. This configuration allows for the processing of both silica membrane-based NucleoSpin® 8/96 and magnetic bead-based NucleoMag® DNA/RNA extraction kits.

Introduction

Plasmid DNA plays a key role in many molecular biology and cell applications. The efficient isolation from bacterial cultures is essential for a variety of molecular applications utilized by many research laboratories. The purity, yield and endotoxin levels of plasmid DNA significantly affect transfection efficiency, cell viability and reproducibility. The main impurities in plasmid DNA purifications derive from endotoxins. These are lipopolysaccharides present in the bacterial cell wall that have cytotoxic effects and are known to influence gene expression in cell cultures, leading to false results in gene expression analysis.

MACHEREY-NAGEL has developed a new generation of silica-membrane-based plasmid DNA purification – NucleoSpin® 96 Plasmid Transfection-grade Plus – that delivers high plasmid yields and extremely low endotoxin levels. This kit combines fast, automated processing with novel endotoxin removal wash buffers, enabling convenient and time saving isolation of transfection-grade DNA (≤ 10 EU/µg with 3 wash steps, ≤ 1 EU/µg with 4 wash steps).

In this collaborative application note we present the automated process on the liquid handling workstation epMotion® 5075vt from Eppendorf using the NucleoSpin® 96 Plasmid Transfection-grade Plus from MACHEREY-NAGEL. The novel optimized protocol allows the processing and managing of 96-well plates facilitating high throughput of samples with low endotoxin levels suitable for transfection experiments.

Products at a glance

NucleoSpin® 96 Plasmid Transfection-grade Plus	
Technology	Silica membrane and endotoxin removal technology
Sample material	Up to 5 mL bacterial culture (*E. coli*, high-copy plasmids)
Target molecules	Plasmid DNA for low endotoxin applications, e. g. transfection of sensitive cells and cell injections
Typical yield	4 – 6 µg/mL *E. coli* culture (depends on the efficiency of plasmid propagation, plasmid copy number, and bacterial cell culture density)
Endotoxin level	≤ 10 EU/µg with 3 washing steps, ≤ 1 EU/µg with 4 washing steps
Preparation time	Approx. 50 min/plate

epMotion® 5075vt	
Technology	Automated liquid handling platform equipped with interchangeable pipetting tools, gripper, heater-shaker, thermo module and integrated vacuum module (further modules are available for different applications).
Software	No-code epBlue 50.2
Sample numbers	1 – 96 samples
Deck positions	Configurable platform with 12 SLAS-/ANSI slots + liquid and solid waste
Pipetting volume	0.2 – 1000.0 µL

Material and methods

The extraction of plasmid DNA was performed using MACHEREY-NAGEL's NucleoSpin® 96 Plasmid Transfection-grade Plus on Eppendorf's epMotion® 5075vt. The verified protocol allows for flexibility in sample numbers up to 96 samples (variable sample number in multiples of 8). Cultivation and harvesting of bacterial cells are recommended to be performed according to the NucleoSpin® 96 Plasmid Transfection-grade Plus user manual. All subsequent steps are performed fully automated on the epMotion® 5075vt without manual intervention. Briefly, bacterial cell pellets from up to 5 mL cultures are resuspended in Resuspension Buffer A1 and subsequently lysed by addition of Lysis Buffer A2 for 5 min at room temperature. Following lysis and neutralization by addition of Buffer A3, the NucleoSpin® 96 Plasmid Transfection-grade Plus kit utilizes two different 96-well filter plates to achieve precise separation as well as high yield and quality of plasmid DNA. Thereby, crude lysates are cleared by the NucleoSpin® 96 Plasmid Filter Plate, removing cellular debris as well as chromosomal DNA. Nucleic acids are subsequently bound to the silica membrane of the NucleoSpin® 96 Plasmid TG Plus Binding Plate during the binding step. Lysate clearance and plasmid DNA binding are performed using vacuum.

The removal of contaminants, such as salts, proteins or endotoxins, from the silica membrane is performed by three washing steps. The integration of a fourth washing step results in even lower endotoxin levels. Finally, highly pure plasmid DNA is eluted under low ionic strength conditions in a slightly alkaline Elution Buffer AE.

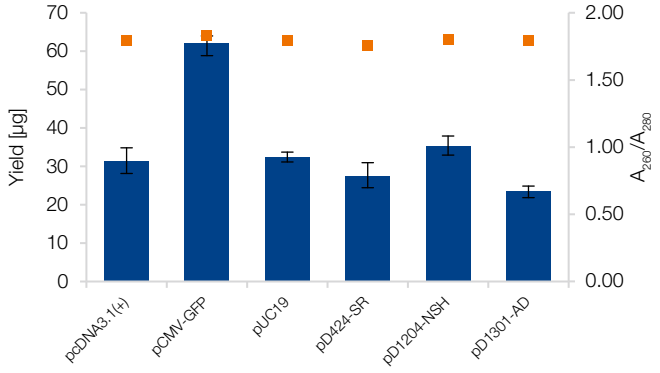
All pipetting and vacuum filtration steps were carried out by the Eppendorf epMotion® 5075vt.

Application data

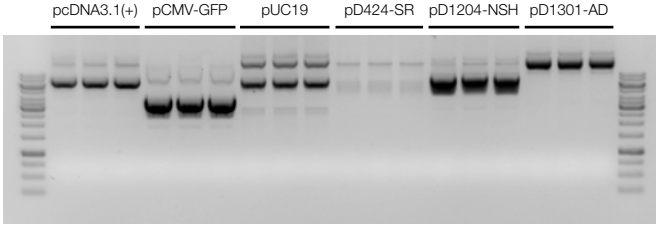
Outstanding yields and purities across different plasmids and bacterial strains

The NucleoSpin® 96 Plasmid Transfection-grade Plus and the epMotion® 5075vt combine the benefits of high-quality, low endotoxin plasmid DNA extractions with a user friendly and automated system. The NucleoSpin® 96 Plasmid Transfection-grade Plus thereby delivers reliable yield and purities for various plasmids from bacterial strains suitable for transfections. Expression vectors for the transfection of mammalian cells (e.g. pcDNA3.1 (+), pD1301-AD, pCMV-GFP), yeast cells (e.g. pD1204-NSH) and bacterial cells (e.g. pD424-SR) consistently deliver high yield and purities.

A)



B)



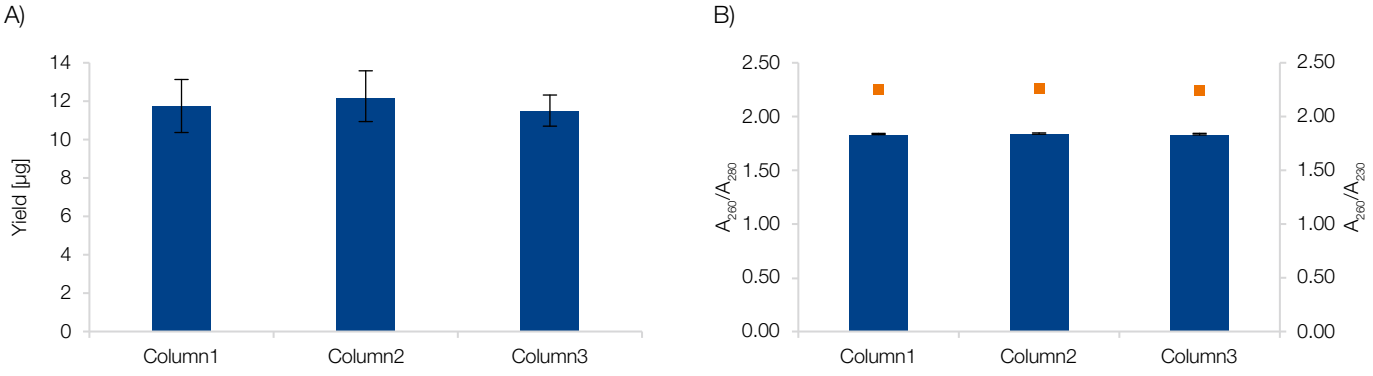
Reproducible yield of plasmid DNA

Plasmid DNA extraction was conducted from six different plasmid constructs and bacterial strains under different growth conditions. *E. coli* Top 10 (pCMV-GFP), *E. coli* NEB 5-α (pD1204-NSH) and *E. coli* NEB Stable (pD1301-AD) were cultivated in 2 mL cultures grown in 96-deep well culture plates. *E. coli* NEB Turbo (pD424-SR) and *E. coli* NEB 10-β (pcDNA 3.1 (+) and pUC19) were cultivated in high density conditions. Automated plasmid DNA extraction was performed on the epMotion® 5075vt using the NucleoSpin® 96 Plasmid Transfection-grade Plus kit. DNA quantity (A, blue bars) and purity (A, Ratio A_{260}/A_{280}, orange squares) were determined photometrically. The reproducibility and DNA integrity were analyzed by gel electrophoresis (B).

High purity of final plasmid DNA is achieved by removal of contaminants, especially endotoxins by the patented Detoxification Buffer ERB. The standard protocol includes three wash steps, resulting in endotoxin levels ≤ 10 EU/µg. The addition of a fourth washing step allows the achievement of endotoxin levels ≤ 1 EU/µg plasmid DNA.

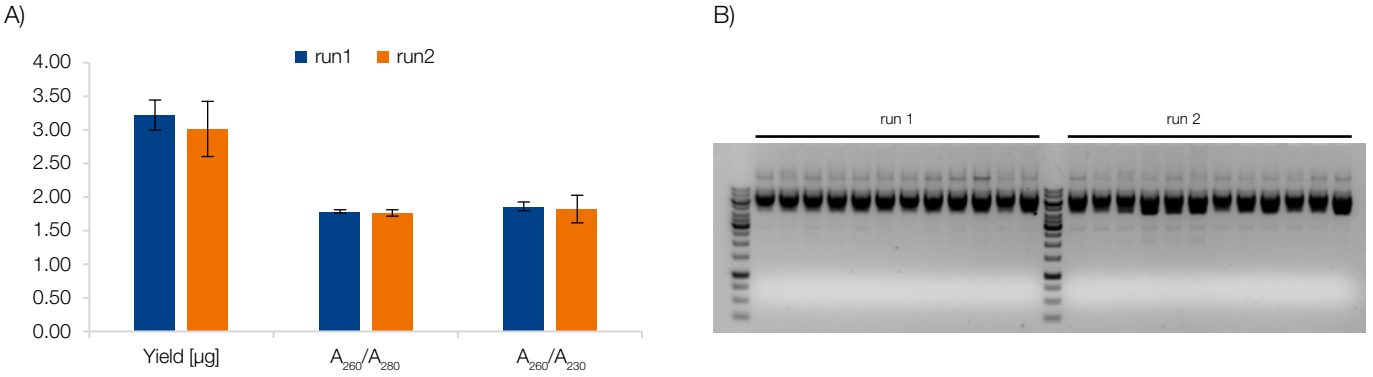
Protocol	Plasmid	Endotoxin [EU/µg DNA]
3 wash steps	pcDNA 3.1(+)	2.0 ± 0.12
4 wash steps	pcDNA 3.1(+)	0.1 ± 0.04

Exceptional reproducibility and repeatability within and between individual runs



Consistent performance within one plate

Plasmid DNA was isolated from 1 mL of *E. coli* NEB 10-ß bacterial culture (high-copy plasmid, pcDNA 3.1, n = 24) using the NucleoSpin® 96 Plasmid TG Plus kit on the Eppendorf epMotion® 5075vt. Bacterial pellets of ODV 10.6 from a flask culture grown in CircleGrowth Media were used for the assessment of yield and purity via UV spectrometry. The results demonstrate exceptional reproducibility and repeatability within individual runs (samples 1 – 24). The bars represent the average yields (A) and purity (B) of three columns of an individual plate (n = 8). The average yield is 11.8 ± 1.22 µg. The average ratio A_{260}/A_{280} is 1.83 (B, blue bars) and the average ratio A_{260}/A_{230} is 2.25 (B, orange dots).



Consitent performance between two runs

Plasmid DNA was isolated from 1 mL of *E. coli* NEB 10-ß bacterial culture (high-copy plasmid, pcDNA 3.1, n = 96) using the NucleoSpin® 96 Plasmid TG Plus kit on the Eppendorf epMotion® 5075vt. The culture was grown in plates in CircleGrowth media. Yield and purity were assessed via UV spectrometry demonstrating exceptional reproducibility and repeatability between two runs (samples 1 – 96). The bars represent the average yields and purity of a full plate (A). The reproducibility and DNA integrity were analyzed by gel electrophoresis (B).

Speed up and automate your transfection-grade plasmid DNA extraction

MACHEREY-NAGEL and Eppendorf deliver a fully automated solution for your high throughput plasmid DNA extraction in transfection-grade purity. We adapted the NucleoSpin® 96 Plasmid Transfection-grade Plus kit on the epMotion® 5075vt to speed up your nucleic acid purification workflow.

- Endotoxin removal wash buffer and optimized filter plates for highly pure plasmid DNA with less than 10 endotoxin units per µg DNA (three wash steps).
- Flexible sample numbers (multiple of 8) and fast processing of 96 samples within 100 minutes (excluding cultivation and harvesting).
- Reliable performance and excellent yields using NucleoSpin® 96 Plasmid Transfection-grade Plus kit on the epMotion® 5075vt.
- Effortless automation and walk-away protocols to save time during your routine tasks and complex workflows.

Application Note NucleoSpin® 96 Plasmid Transfection-grade Plus

Eppendorf and MACHEREY-NAGEL as strong collaboration partners

Download Application Notes

Scan the QR code or click the link below to read more about the collaboration between MACHEREY-NAGEL and Eppendorf and read more about our scripts and methodologies of our DNA, RNA and protein purification solutions on Eppendorf's epMotion® systems.

www.mn-net.com/eppendorf

Evolve your liquid handling journey with the epMotion®

For more information on automation solutions with liquid handling systems, scan the QR code or click the link below.

www.eppendorf.com/own-your-solution/automated/

Ordering information

Product	Specifications	Pack of	REF
NucleoSpin® 96 Plasmid Transfection-grade Plus	Silica membrane-based kit for the isolation of ultrapure plasmid DNA from bacterial cultures with endotoxin levels ≤ 1 EU/µg plasmid DNA, including NucleoSpin® Plasmid TG Plus Binding Plates, NucleoSpin® Filter Plate, Culture plates, gas permeable foils, elution plates, wash plates, buffers, and RNase A (lyophilized)	1 × 96 / 4 × 96 preps	740501.1 / .4
NucleoSpin® 96 Plasmid Transfection-grade Plus Binding Plate	96-well plates for the isolation of plasmid DNA	24 plates	740501.24S
Buffer ERB	Detoxification Buffer ERB for transfection-grade Plasmid DNA Isolation	500 / 1000 mL	740495.500 / .1000
Eppendorf epMotion® 5075vt*	Automated liquid handling platform including MultiCon PC, a completely self-contained housing, vacuum system with accessories, gripper, ThermoMixer®, epBlue software, keyboard, mouse, system for solid and liquid waste, 100 – 240 V/50 – 60 Hz		5075 000 044*

*For more detailed information, please visit www.eppendorf.com. To contact Eppendorf SE, please email eppendorf@eppendorf.com or visit www.eppendorf.com/contact.

Want to schedule an e-demo?
Need additional information?

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