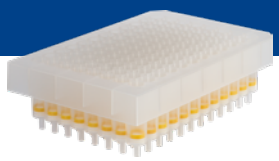


NucleoSpin® 96 Plasmid Transfection-grade Plus

Automated high throughput plasmid DNA purification using silica membrane-based kits with Waters™ liquid handling pipetting robot Andrew+™ and Extraction+™ vacuum module

Application benefits

Elevate your nucleic acid purification processes using MACHEREY-NAGEL's DNA/RNA purification kits in conjunction with Waters Andrew+ automated 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
- Optimize speed while minimizing plastic consumption for maximum efficiency
- For any questions regarding reagents or automation support please contact: support@mn-net.com

Keywords

Plasmid DNA, nucleic acid extraction, automated plasmid DNA purification, *E. coli* culture, liquid handling system, miniprep, high-purity, high-yield, transfection-grade, endotoxin

Andrew+

The Andrew+ offers versatility through the incorporation of various modules, pipettes, gripper and vacuum chamber. The configuration allows for the processing of silica-membrane-based NucleoSpin® 96 DNA/RNA as well as NucleoBond® Midi extraction kits.

Introduction

Cloning, genetic engineering, gene expression, transfection, protein production and vaccine development studies represent a subset of the diverse applications underscoring the significance of plasmid DNA across various domains of biological research. The achievement of high-quality plasmid DNA characterized by consistent yields and purity is imperative for the successful execution of these applications.

The main impurities in plasmid DNA purifications derive from endotoxins. Endotoxins are lipopolysaccharides derived from the bacterial cell wall that have cytotoxic effects and negatively influence cell viability and transfection efficiency. Additionally, endotoxins are known to influence gene expression in cell cultures, leading to false results in gene expression analysis. The efficient isolation of plasmid DNA from bacterial cultures is essential for a variety of molecular applications utilized by many research laboratories.

MACHEREY-NAGEL has developed a new generation of silica-membrane-based plasmid DNA purification – NucleoSpin® 96 Plasmid Transfection-grade Plus – that deliver 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 cooperation with Waters pipetting robot Andrew+ with the Extraction+ module as vacuum chamber, MACHEREY-NAGEL's NucleoSpin® 96 Plasmid Transfection-Grade Plus purification enables the vacuum processing of 96-well plates. This novel and optimized protocol allows high throughput plasmid DNA purifications with low endotoxin levels suitable for transfection experiments.

Products at a glance

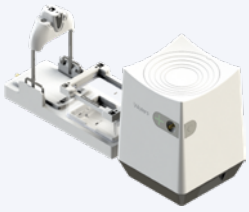
NucleoSpin® 96 Plasmid Transfection-grade Plus	
Technology	Silica membrane and endotoxin removal technology
Sample material	Up to 5 mL bacterial culture (*E. coli*, high-copy, plasmids)
Target molecules	Plasmid DNA for low endotoxin applications, e. g. transfection of sensitive cells and cell injections
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

Andrew+	
Technology	Automated liquid handling utilizing wireless connected electronic pipettes and a dynamic deck configuration consisting of up to 11 dominos on a conventional bench space. Protocol execution via the OneLab™ cloud software.
Sample numbers	1 – 96 samples
Deck position	For vacuum applications using the Extraction+ module consisting of a remote connected vacuum pump, manifold for SPE processing and gripper.

Application Note NucleoSpin® 96 Plasmid Transfection-grade Plus

NucleoSpin® 96 kits now automated on Andrew+ with the Extraction+ vacuum chamber module

- Verified protocols
- Step-by-step protocol in OneLab
- Rapid and easy nucleic acid purifications
- High plasmid DNA yields with low endotoxin levels



Extraction+ module

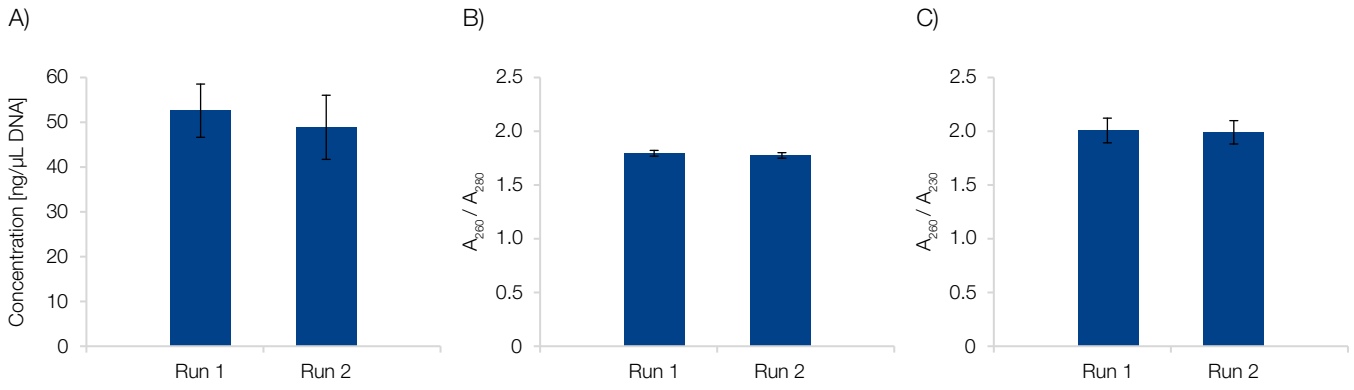
Material and methods

The extraction of plasmid DNA was carried out utilizing MACHEREY-NAGEL's NucleoSpin® 96 Plasmid Transfection-grade Plus kit on an Andrew+ platform using the vacuum module Waters Extraction+. The biologically-verified protocol allows for flexibility in sample numbers, accommodating 8 – 96 plasmid purifications per run. Briefly, after pelleting bacterial cultures, cell resuspension, alkaline lysis, and neutralization steps are performed by the Andrew+ using the Shaker+ orbiting shaking module. Neutralized crude lysates are transferred to the NucleoSpin® 96 Plasmid Filter Plate and filtered via vacuum.

After a brief user intervention to discard the NucleoSpin® 96 Filter Plate and place the NucleoSpin® 96 Plasmid TG Plus Binding Plate on the manifold the lysate is transferred and the binding is performed on the Extraction+. After binding plasmid DNA to the silica membrane, contaminants such as salts, proteins, or endotoxins were removed through three consecutive washing steps. The highly purified plasmid DNA was ultimately eluted under low ionic strength conditions using a slightly alkaline elution buffer. All pipetting steps were carried out by the Andrew+, utilizing the Extraction+ module for all vacuum filtration steps.

Application data

High reproducibility with the NucleoSpin® 96 Plasmid Transfection-grade Plus kit



High reproducibility of plasmid DNA extraction within and in between runs

Plasmid DNA was isolated from 1 mL of *E. coli* Top 10 bacterial culture (high-copy plasmid, pcDNA 3.1(+), n = 96) using the NucleoSpin® 96 Plasmid TG Plus kit on the Andrew+. Yield (A) and Purity (Ratio A_{260}/A_{280}: B, Ratio A_{260}/A_{230}: C) were assessed via UV spectrometer demonstrating exceptional reproducibility between two runs (n = 96).

Maximal flexibility for plasmid preparations with the Andrew+

- Perform high throughput mini preparations using the Extraction+ module
- Perform large-scale NucleoBond® Xtra Midi preparations on the Andrew+

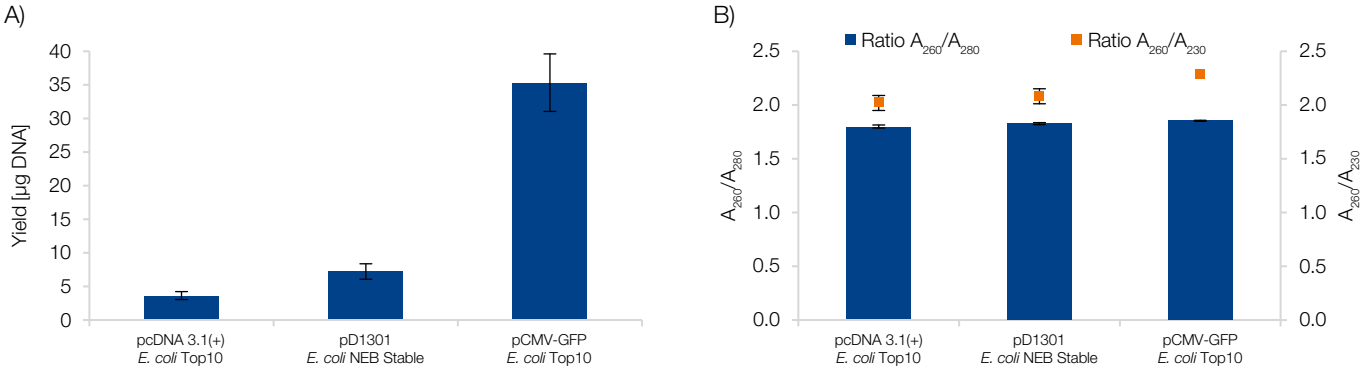
MACHEREY-NAGEL and Andrew+ – the perfect match for automated high-throughput and large-scale plasmid preparations

Application Note NucleoSpin® 96 Plasmid Transfection-grade Plus

Outstanding yields and purities across different plasmids

The NucleoSpin® 96 Plasmid Transfection-grade Plus and the Andrew+ combine the benefits of high-quality, low endotoxin plasmid DNA extractions with a user-friendly and automated system.

The combination is suitable for a wide range of plasmid constructs, plasmid concentrations and cultures resulting in high yields and purities.



High plasmid DNA yields and purities across different plasmids and bacterial strains

Plasmid DNA extraction was conducted from three different plasmid constructs and bacterial strains under different growth conditions. *E. coli* Top 10 (pcDNA 3.1(+) and pCMV-GFP, mammalian expression vectors) and *E. coli* NEB Stable (pD1301-AD, mammailian expression vector with Cas9-nuclease gene) were cultivated in high density conditions. Automated plasmid DNA extraction was performed on the Andrew+ using the NucleoSpin® 96 Plasmid Transfection-grade Plus kit. DNA yield (A, blue bars) and purity (B, A_{260}/A_{280}, blue bars, A_{260}/A_{230}, orange squares) were determined photometrically.

Reduced endotoxin levels

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 that result in endotoxin levels ≤ 10 EU/µg. The addition of a fourth washing step allows the achievment of endotoxin levels ≤ 1 EU/µg plasmid DNA. In this table, endotoxin levels were measured after three washing steps showing low endotoxin levels suitable for transfections.

Culture	Plasmid	ODV	Yield [µg]	A_{260}/A_{280}	A_{260}/A_{230}	Endotoxin [EU/µg DNA]
E. coli Top10	pcDNA 3.1(+)	10	3.62 ± 0.59	1.79 ± 0.01	2.02 ± 0.07	9.9 ± 2.97
E. coli NEB Stable	pD1301	10	7.24 ± 1.16	1.83 ± 0.01	2 07 ± 0.01	1.9 ± 1.03
E. coli Top10	pCMV-GFP	10	35.38 ± 4.23	1.85 ± 0.00	2.28 ± 0.02	0.5 ± 0.37

Speed up and automate your transfection-grade plasmid DNA extraction

MACHEREY-NAGEL and Andrew+ deliver an 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 Andrew+ using the Extraction+ vacuum module 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.
- 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 Andrew+ robot.

Application Note NucleoSpin® 96 Plasmid Transfection-grade Plus

Andrew+ and MACHEREY-NAGEL as strong collaboration partners

The combined expertise of MACHEREY-NAGEL and the Andrew+ robot allowed the establishment of fully automated solutions for high-quality nucleic acid purifications. MACHEREY-NAGEL's NucleoSpin® and NucleoBond® technology enable rapid and easy nucleic acid purificiations. Waters OneLab software offers a complete on-screen, step by step guide through the procotol and allows to track each protocol step. Together, we have established several solutions for fully automated genomic DNA extraction and plasmid DNA extractions from Mini to Midi formats.

For more information, please have a look at our partner pages.

www.mn-net.com/andrew

www.waters.com/labautomation

Want to schedule an e-demo?
Need additional information?
Contact the MN technical support!
support@mn-net.com
+49 (0) 2421 969 333

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	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
Andrew+ Pipetting Robot*		1	
Extraction+ module*		1	176005201

* For more detailed information, please visit *onelab.com/library*.
To contact Waters, please visit www.waters.com/labautomation.

KATEN300281 AN NucleoSpin® 96 plasmid transfection grade plus en2a

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