NucleoSpin® 96 Plasmid

Automated high throughput plasmid DNA purification using silica membrane-based kits with Waters™ liquid handling 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
- 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

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

Genetic engineering, protein production and pharmaceutical advancements all require plasmid DNA as a starting material. The extraction of plasmid DNA from bacterial cultures therefore plays a pivotal role for various applications in molecular biology laboratories. While being one of the most common techniques used in molecular laboratories, the extraction of plasmid DNA is time-consuming. The automation of this process provides significant advances in efficiency and reliability, particularly in downstream applications where consistent, high-quality results are essential. By automating plasmid extraction, researchers can considerably reduce hands-on time and increase their reproducibility.

MACHEREY-NAGEL's NucleoSpin® 96 Plasmid kit offers an established method for the silica-membrane based parallel extraction of 96 samples resulting in high plasmid yields and purities. The extracted plasmid DNA can directly be used for molecular approaches including sequencing and cloning. This kit combines fast, automated processing with established and reliable plasmid purifications resulting in molecular-cloning grade purity (> 50 EU/µg).

In cooperation with Waters pipetting robot Andrew+ with the Extraction+ module as vacuum chamber, MACHEREY-NAGEL's NucleoSpin® 96 Plasmid kit enables the automated vacuum processing of 96-well plates. This novel and optimized protocol allows high throughput plasmid DNA purifications with high DNA yields.

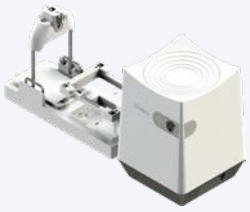
Products at a glance

NucleoSpin® 96 Plasmid	
Technology	Silica membrane technology
Sample material	Up to 5 mL bacterial culture (*E. coli*, high-copy, plasmids)
Target molecules	Plasmid DNA for standard applications, e.g. genetic engineering, molecular cloning (including PCR, restriction analysis, sequencing), transformation
Typical yield	4 – 30 µg/mL *E. coli* culture (depends on the efficiency of plasmid propagation, plasmid copy number, and bacterial cell culture density)
Endotoxin level	> 50 EU/µg DNA
Preparation time	Approx. 90 – 100 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™ 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.

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 the purity levels you require



Extraction+ module

Material and methods

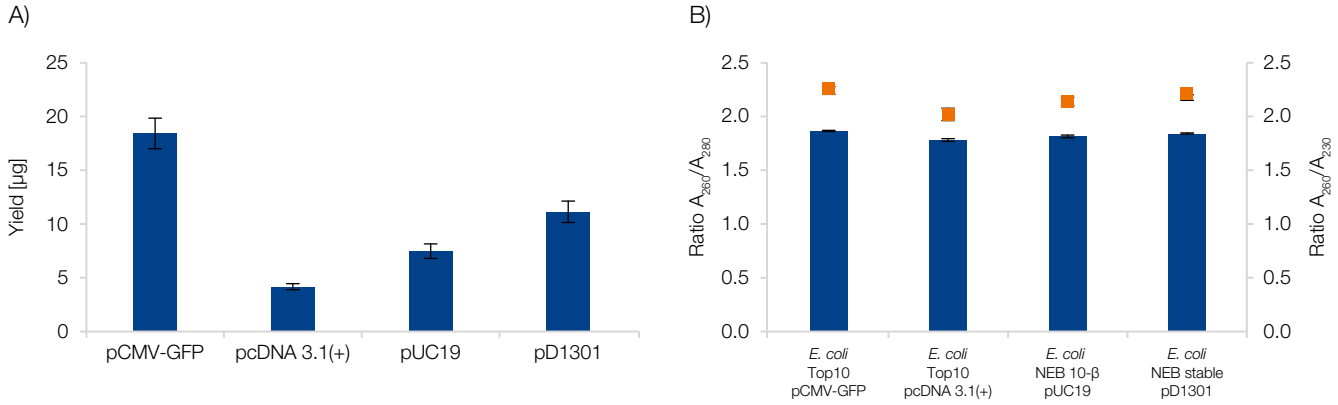
The extraction of plasmid DNA was carried out utilizing MACHEREY-NAGEL's NucleoSpin® 96 Plasmid kit on an Andrew+ platform using the vacuum module Waters Extraction+. The biologically-verified protocol allows for flexibility in sample numbers, accommodating 8 – 96 plasmid purifications per run. Briefly, 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 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

Outstanding yields and purities across different plasmids

The NucleoSpin® 96 Plasmid and the Andrew+ combine the benefits of high-quality 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

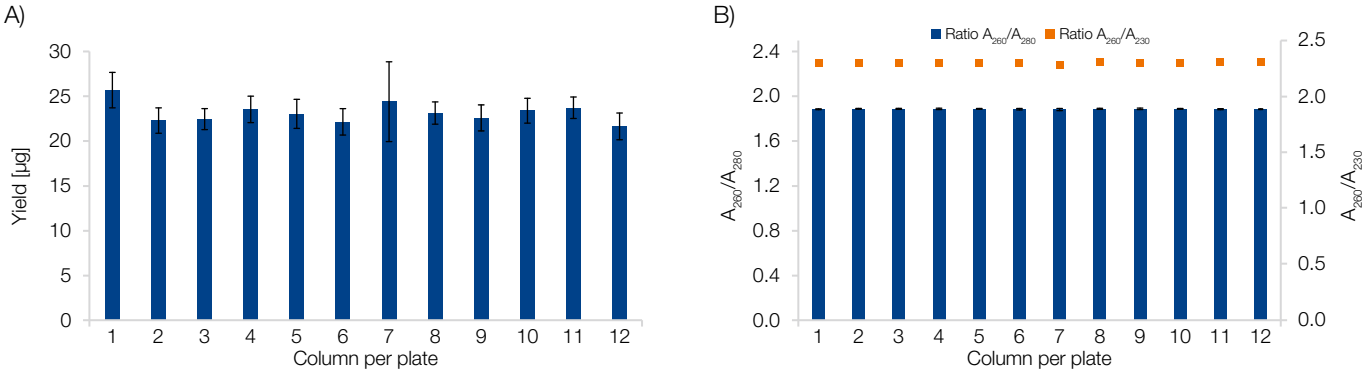
Plasmid DNA extraction was conducted from four different plasmid constructs and bacterial strains under different growth conditions. *E. coli* Top 10 (pcDNA 3.1(+) and pCMV-GFP, mammalian expression vectors), *E. coli* NEB 10-β (pUC19) and *E. coli* NEB Stable (pD1301-AD, mammalian expression vector with Cas9-nuclease gene) were cultivated in high density conditions. Automated plasmid DNA extraction was performed on the Andrew+ using the NucleoSpin® 96 Plasmid kit. DNA yield (A, blue bars) and purity (B, A_{260}/A_{280}, blue bars, A_{260}/A_{230}, orange squares) were determined photometrically.

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+ via gravity flow

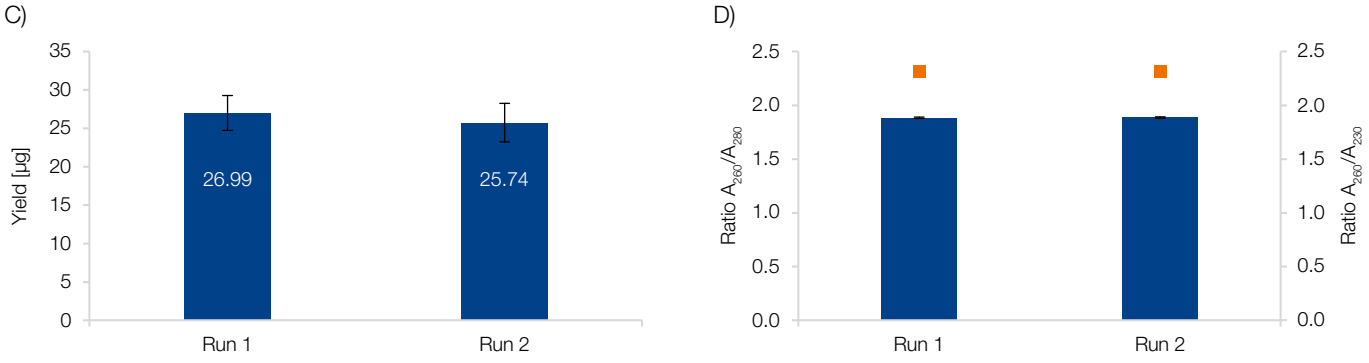
MACHEREY-NAGEL and Andrew+ – the perfect match for automated high-throughput and large-scale plasmid preparations

Exceptional reproducibility and repeatability within and between individual runs



Consistent performance within one plate

Plasmid DNA extraction was isolated from 1 ml *E. coli* Top 10 bacterial culture (ODV = 10, high-copy plasmid, pCMV-GFP, n = 96) using the NucleoSpin® 96 Plasmid kit on the Andrew+. Yield (A) and Purity (B, Ratio $A_{260}/_{280}$: blue bars, Ratio A_{260}/A_{230}: orange squares) were assessed via UV spectrometer demonstrating exceptional reproducibility within one run. The bars and squares represent the average of 12 columns of an individual plate (n = 8). The average yield is 23.18 ± 1.7 µg. The average ratio A_{260}/A_{280} is 1.89. The average A_{260}/A_{230} ratio is 2.3.



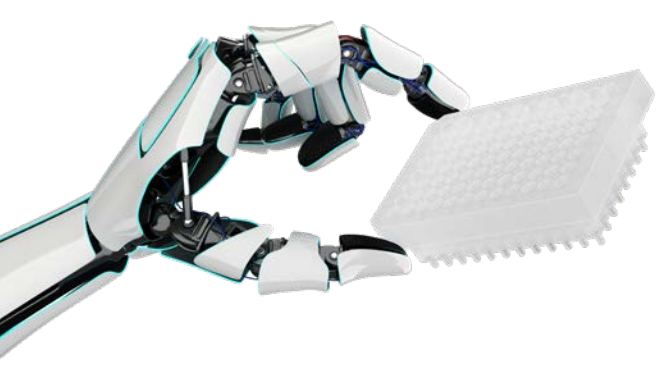
Consistent performance between two runs

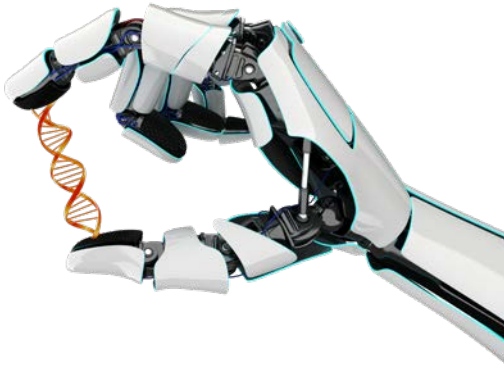
Plasmid DNA was isolated from *E. coli* NEB Top 10 bacterial culture (ODV = 10, high-copy plasmid, pCMV-GFP, n = 48) using the NucleoSpin® 96 Plasmid kit on Andrew+. Yield (C) and purity (D, Ratio $A_{260}/_{280}$: blue bars, Ratio A_{260}/A_{230}: orange squares) were assessed via UV spectrometry demonstrating exceptional reproducibility and repeatability between two runs (n = 48). The bars represent the average yields and purity of half a plate. The ratio between both runs was > 95.37 %

Speed up and automate your plasmid DNA extraction

MACHEREY-NAGEL and Andrew+ deliver an automated solution for your high throughput plasmid DNA extraction in molecular purity. We adapted the NucleoSpin® 96 Plasmid kit on the Andrew+ using the Extraction+ vacuum module to speed up your nucleic acid purification workflow.

- 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 kit on the Andrew+ robot.





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 purifications. Waters OneLab software offers a complete on-screen, step by step guide through the protocol 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
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Ordering information

Product	Specifications	Pack of	REF
NucleoSpin® 96 Plasmid	Silica membrane-based kit for the isolation of ultrapure plasmid DNA from bacterial cultures with endotoxin levels > 50 EU/µg plasmid DNA, including NucleoSpin® Plasmid Binding Plates, NucleoSpin® Filter Plate, Culture plates, gas permeable foils, elution plates, wash plates, buffers, and RNase A (lyophilized)	1 × 96 / 4 × 96 / 24 x 96	740625.1 / .4 / .24
Andrew+ Pipetting Robot*	Automated liquid handling system for precise and reproducible pipetting workflows	1	176005081
Extraction+ module*	Vacuum-based module enabling automated filtration and nucleic acid purification	1	176005201
Shaker+ module*	Orbital shaking module for mixing and incubation during sample preparation	1	176004577
Culture plate	Square-well block with 2.1 mL square wells used for growing, harvesting or lysing of bacterial cultures, with Gas-permeable foil	4 sets / 24 sets	740488 / .24
MN wash plate	Plate to minimize the risk of cross-contamination	4 / 24	740479 / .24
Elution plate U-bottom	96-well microplate with 300 µl u-bottom, including self-adhering foil	24	740486.24

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

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