MACHEREY-NAGEL

NucleoSpin® 96 Plasmid Transfection-grade

Automated purification of transfection-grade plasmid DNA using Hamilton® $[MPE]^2$

Introduction

The efficient isolation of plasmid DNA from bacterial cultures is essential for a variety of molecular applications utilized by many research laboratories. Transfection of cultured cells is one of the most common applications for isolated plasmids and requires highly pure DNA. The main impurities in plasmid DNA preparations derive from endotoxins. Endotoxins are lipopolysaccharides 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.

MACHEREY-NAGEL has developed a 96-well kit, NucleoSpin® 96 Plasmid Transfection-grade, for the isolation of endotoxin reduced plasmid DNA based on silica membrane technology. The kit combines a very fast processing with novel endotoxin removal wash buffers, enabling convenient and time saving isolation of transfection-grade DNA (≤ 50 EU/µg DNA, endotoxin units per µg DNA).

Here we present the first implementation of the NucleoSpin® 96 Plasmid Transfection-grade kit on a positive pressure unit using the $[MPE]^2$ positive pressure module from Hamilton. The $[MPE]^2$ module maintains equal pressure across the NucleoSpin® Plasmid Binding Plates eliminating the possibility of uneven flow through. Our optimized protocol allows the processing of 96 samples within approximately 60 to 90 minutes, depending on platform setup.

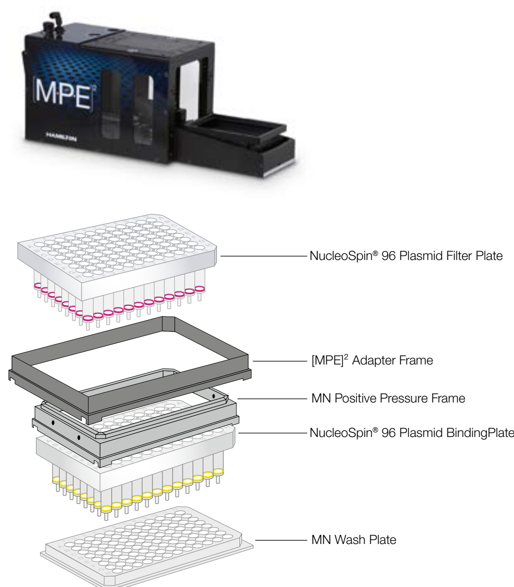
Product at a glance

NucleoSpin® 96 Plasmid Transfection-grade	
Technology	Silica membrane technology
Sample material	Up to 5 mL bacterial culture (*E. coli*, high-copy plasmids)
Preparation time	Approx. 60–90 min depending on platform setup
Typical yield	5–20 µg
Elution volume	100–200 µL
Theoretical binding capacity	20 µg

$[MPE]^2$	
Technology	Monitored Multi-flow, Positive Pressure Evaporative Extraction
Sample volume	Optional reagent fill module with up to 15 reagent bottles
Capacity	24 / 48 / 96 samples
Size / weight	44.5 x 15.9 x 18.1 cm / 6.9 kg

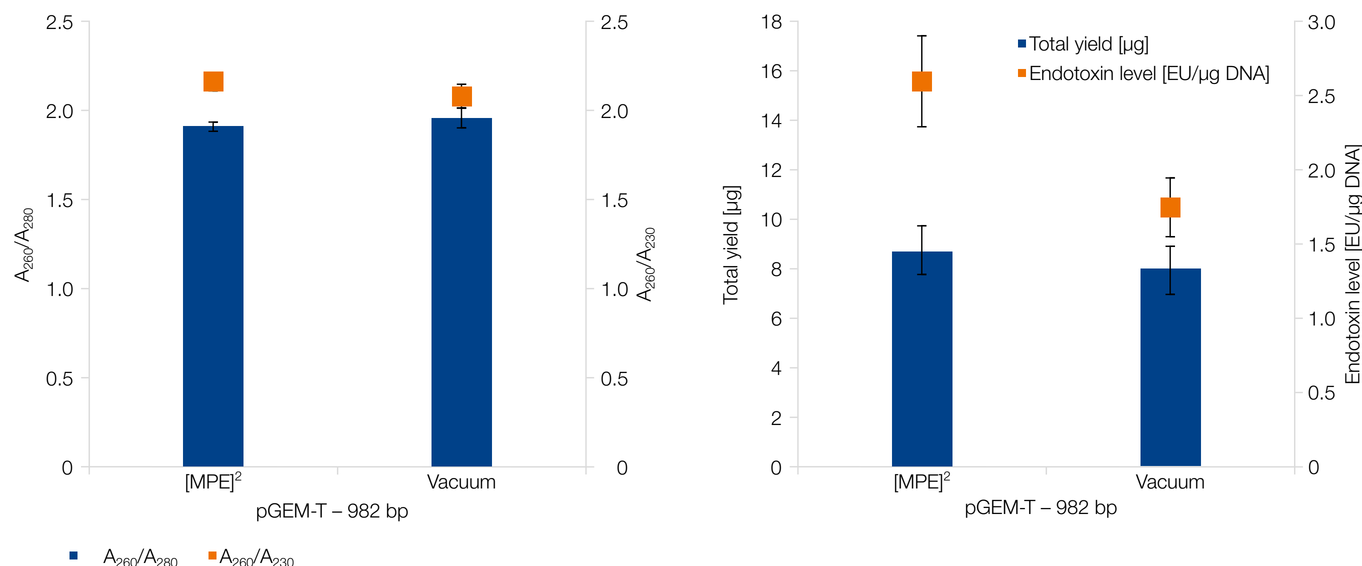
Material and methods

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, all subsequent steps are performed on the $[MPE]^2$ positive pressure module.



Crude lysates are cleared by the NucleoSpin® Plasmid Filter Plate, removing cellular debris as well as chromosomal DNA. Nucleic acids are then reversibly bound to the silica membrane of NucleoSpin® Plasmid Binding Plate during the binding step. A special MN positive pressure frame allows the direct filtration into the NucleoSpin® Plasmid Binding Plate without additional intervention. Endotoxins and proteins are removed by the innovative Detoxification Buffer ERB. Further contaminations such as salts or proteins are subsequently removed with ethanolic Buffer AQ while traces of ethanol are removed by positive pressure. Pure plasmid DNA is eluted under low ionic strength conditions with slightly alkaline Buffer AE (5 mM Tris/HCl, pH 8.5) and is ready for any common downstream application including transfection (research use only).

Application data



Purity of isolated plasmid DNA from bacterial cultures

E. coli DH5α™, transformed with a plasmid containing a 982 bp inserts, was used to isolate plasmid DNA from 1.5 mL of bacterial cultures (high-copy plasmid pGEM®-T Easy; n = 24) using the NucleoSpin® 96 Plasmid Transfection-grade kit on a positive pressure module ([MPE]²) or a manual vacuum manifold (vacuum). Purity was determined by UV spectrometry via A_{260}/A_{280} (dark blue bar) and A_{260}/A_{230} (orange squares) revealing comparable quality of positive pressure ([MPE]²) or vacuum processed (Vacuum) samples

Reproducibility test for yield and purity in 96-well plate format	
Total yield [µg]	6.41 µg ± 0.69
Ratio A_{260}/A_{280}	1.90 µg ± 0.023
Ratio A_{260}/A_{230}	2.04 µg ± 0.051

Determination of reproducibility for 96 samples

The reproducibility of high copy plasmid DNA isolation from 1.5 mL of an *E. coli* cultures was analyzed using 96 samples. Total yield was determined and purity was analyzed by UV spectrometry via A_{260}/A_{280} and A_{260}/A_{230}.

Isolation of plasmid DNA from bacterial cultures and measurement of endotoxin levels

E. coli DH 5α™, transformed with a plasmid containing a 982 bp inserts, was used to isolate plasmid DNA from 1.5 mL of bacterial cultures (high-copy plasmid pGEM®-T Easy; n = 24) using the NucleoSpin® 96 Plasmid Transfection-grade kit on a positive pressure module ([MPE]²) or a manual vacuum manifold (Vacuum). Total yield (dark blue bars) was determined by UV spectrometry showing comparable results between positive pressure or vacuum processed samples. The Endotoxin level (orange squares) was determined by a quantitative chromogenic LAL-test (n=4) to assess the EU (Endotoxin Units) per µg DNA.

Automate your plasmid DNA extraction

MACHEREY-NAGEL and Hamilton deliver a sophisticated solution for your high throughput tranfection-grade Plasmid DNA extraction. The NucleoSpin® 96 Plasmid Transfection-grade kit procedure can be easily adapted for the [MPE]² positive pressure module to speed up your extraction workflow.

- Reliable performance and excellent yields using NucleoSpin® 96 Plasmid Transfection-grade on the [MPE]² positive pressure module
- Low endotoxin levels comparable to standard vacuum processing
- Compact and automated plasmid isolation of 96 samples in 60–90 minutes

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® 96 Plasmid Transfection-grade	Kit based on silica membrane technology for the isolation of transfection-grade plasmid DNA from bacterial cultures in 96-well format	1 x 96 / 4 x 96 / 24 x 96	740491.1 / .4 / .24
MN Positive Pressure Frame	Adaptor for direct filtration using the NucleoSpin® 96 Plasmid kits	1 piece	740474
MN Wash plate	Plate to minimize the risk of cross-contamination	4 sets 24 sets	740479 740479.24
[MPE]²	Monitored multi-flow, positive pressure evaporative extraction module with 96 air manifold and evaporator		96160–04*

NucleoSpin® is a registered trademarks of MACHEREY-NAGEL; [MPE]2 is a trademark of Hamilton®; DH5α™ is a Trademark of Thermo Fisher Scientific.

* For more detailed information, please visit www.hamiltoncompany.com/robotics. To find a Hamilton subsidiary or distributor in your area, please visit www.hamiltoncompany.com/contacts.

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