MACHEREY-NAGEL

NucleoSpin® 96 Plasmid

Automated purification of plasmid DNA using the Hamilton [MPE]^2 positive pressure module

Introduction

The efficient isolation of plasmid DNA from bacterial cultures is essential for a variety of molecular applications utilized by many research laboratories. MACHEREY-NAGEL designed the NucleoSpin® 96 Plasmid kit for the reliable and automated parallel purification of plasmid DNA from bacterial cultures in a 96-well format. High quality plasmid DNA can be extracted and directly used for subsequent applications such as cloning or sequencing. This versatile silica membrane based kit is compatible with either vacuum or centrifugation processing and performs well in both manual and automated formats.

MACHEREY-NAGEL is continuously expanding its collaborations with automation partners in order to offer more support to high throughput customers. We now present the first implementation of the NucleoSpin® 96 Plasmid 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 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.

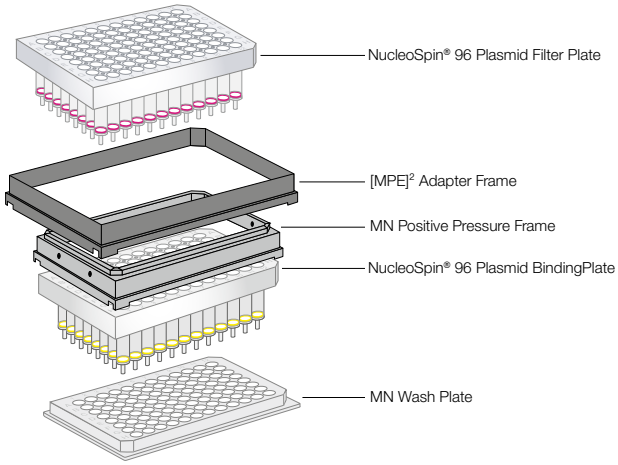
Products at a glance

NucleoSpin® 96 Plasmid	
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	4–6 µg/mL *E. coli* culture
Elution volume	100–200 µL
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

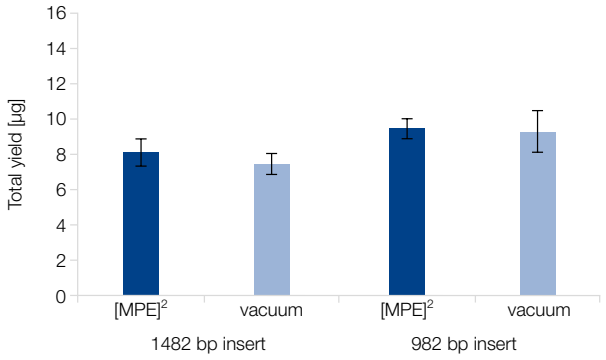
Material and methods

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.



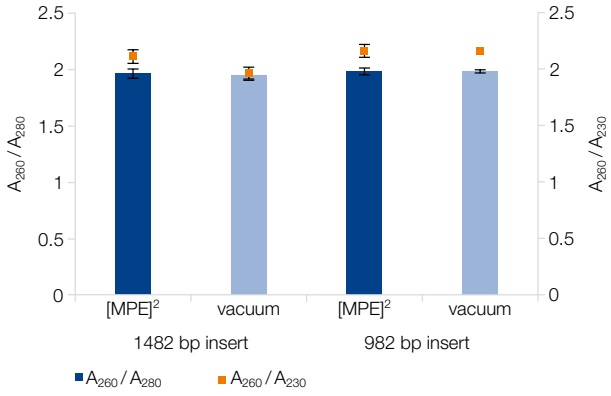
The NucleoSpin® 96 Plasmid kit utilizes two different 96-well filter plates in order to achieve a precise separation as well as high yield and quality of plasmid DNA. Crude lysates are cleared by the NucleoSpin® 96 Plasmid Filter Plate, removing cellular debris as well as chromosomal DNA. Nucleic acids are subsequently bound to the silica membrane of the NucleoSpin® 96 Plasmid Binding Plate during the binding step. MACHEREY-NAGEL developed a special adaptor (MN Positive Pressure Frame) allowing the direct filtration from the NucleoSpin® 96 Plasmid Filter Plate into the NucleoSpin® 96 Plasmid Binding Plate without additional intervention. Moreover it is recommended to use the MN Wash Plate Plate underneath the plate stack setup to minimize the risk of cross-contamination. Contaminants, such as salts or proteins, are then removed from the silica membrane by three washing steps, and highly pure plasmid DNA is finally eluted under low ionic strength conditions in a slightly alkaline Elution Buffer AE.

Application data



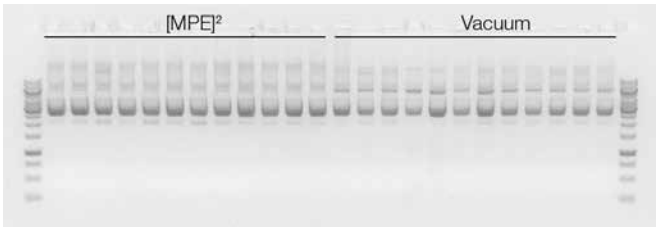
Isolation of plasmid DNA from bacterial cultures

Plasmid DNA of two different bacterial strains, transformed with plasmids containing either a 1482 bp or a 982 bp inserts, was isolated from 1.5 mL of bacterial cultures (*E. coli* DH 5α™, high-copy plasmid pGEM®-T Easy; n = 24) using the NucleoSpin® 96 Plasmid kit on a [MPE]^2 positive pressure module (dark blue) or a manual vacuum manifold (light blue). Total yield was determined by UV spectrometry showing comparable yields between positive pressure or vacuum processed samples.



Purity of isolated plasmid DNA from bacterial cultures

Plasmid DNA of two different bacterial strains, transformed with plasmids containing either a 1482 bp or a 982 bp inserts, was isolated from 1.5 mL of bacterial cultures (*E. coli* DH 5α, high-copy plasmid pGEM®-T Easy; n = 24) using the NucleoSpin® 96 Plasmid kit on a [MPE]^2 positive pressure module (A_{260}/A_{280}: dark blue bars; A_{260}/A_{230}: orange squares) or a manual vacuum manifold (A_{260}/A_{280}: light blue bars; A_{260}/A_{230}: orange squares). Purity was determined by UV spectrometry revealing comparable quality of positive pressure or vacuum processed samples.



Reproducible yields of plasmid DNA

The reproducibility of high copy plasmid DNA isolated from 1.5 mL of an *E. coli* cultures was analyzed by gel electrophoresis (10 µL per eluate; 1 % TAE gel; Marker: GeneRuler™ 1 kb DNA Ladder – Thermo Scientific)

Automate your plasmid DNA extraction

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

- Reliable performance and excellent yields using NucleoSpin® 96 Plasmid on the [MPE]^2 positive pressure module
- Compact automated processing of 96 samples in 60–90 minutes
- Optimized workflow by using the novel MN Positive Pressure Frame in combination with the MN Wash Plate

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® 96 Plasmid	Kit based on silica-membrane technology for the isolation of plasmid DNA from bacterial cultures in 96-well format	1 x 96 4 x 96 24 x 96	740625.1 740625.4 740625.24
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 740488.24
MN Wash plate	Plate to minimize the risk of cross-contamination	4 24	740479 740479.24
MN Positive Pressure Frame	Adaptor frame for the direct filtration of crude lysate from NucleoSpin Filter Plates into NucleoSpin Binding Plates	Pack of 1	740474
Elution plate U-bottom	96-well microplate with 300 µL u-bottom, including self-adhering foil	24	740486.24
[MPE]^2 *	Monitored multi-flow, positive pressure evaporative extraction module with 96 air manifold and evaporator		96160-04

*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.