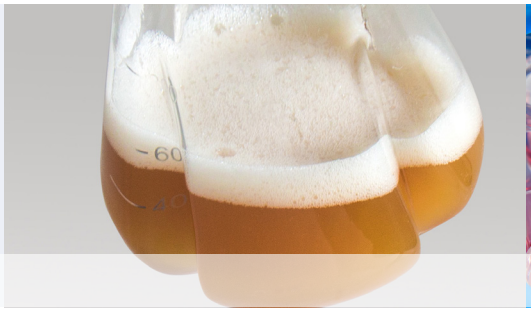
MACHEREY-NAGEL

NucleoSpin® 96 Plasmid Transfection-grade

Purification of plasmid DNA with transfection-grade purity using the platform ep*Motion*® 5075vt



Introduction

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 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 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 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).

This application note describes the automated process on the liquid handling workstation ep*Motion*® 5075vt using the NucleoSpin® 96 Plasmid Transfection-grade kit from MACHEREY-NAGEL. The novel optimized protocol allows the processing of a variable sample number in multiples of 8 (8–96). The processing of 96 samples takes approximately 100 minutes excluding cultivation and harvesting.

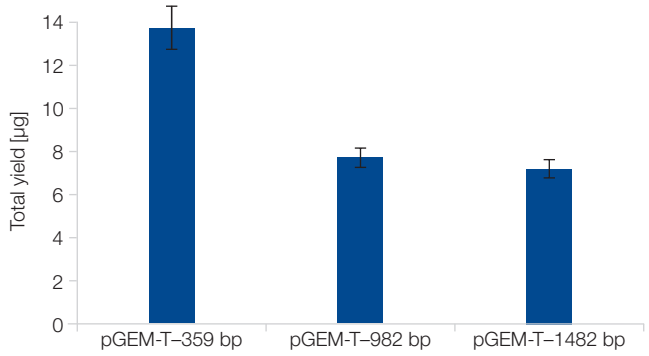
Product at a glance

NucleoSpin® 96 Plasmid Transfection-grade	
Technology	Silica membrane and endotoxin reduction technology
Sample material	Up to 5 mL bacterial culture (*E. coli*, high-copy plasmids)
Preparation time	Approx. 100 min for 96 samples (excluding cultivation and harvesting).
Format	Variable sample number in multiples of 8 (8–96)
Typical yield	5–20 µg
Elution volume	100–200 µL
Binding capacity	20 µg

Material and methods

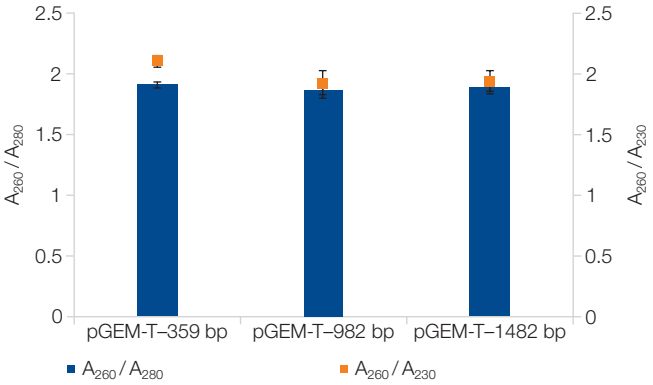
The optimized protocol is programmed to process up to 96 samples in parallel (Variable sample number in multiples of 8) and developed for the ep*Motion*® 5075vt platform. Cultivation and harvesting of bacterial cells is recommended to perform according to the NucleoSpin® 96 Plasmid Transfection-grade user manual. 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 ep*Motion*® 5075vt. 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. Lysate clearance and Plasmid DNA binding is performed by vacuum. 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. 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



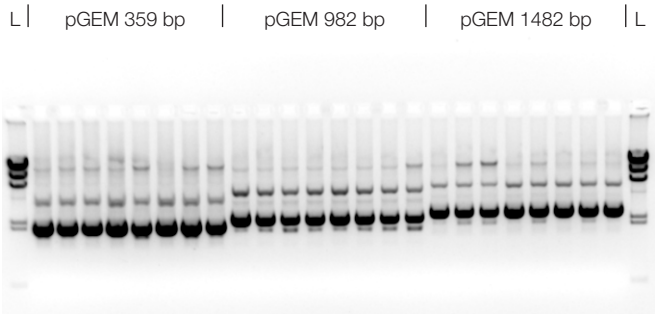
Isolation of transfection-grade plasmid DNA from bacterial cultures

Plasmid DNA of three different bacterial strains, transformed with plasmids containing either a 1482 bp, a 982 bp or a 359 bp insert, 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 Transfection-grade kit on the ep*Motion*® 5075vt. Total yield was determined by UV spectrometry (dark blue bars). All measured endotoxin contents showed significant less than 50 EU/µg DNA (EU = endotoxin units)



Purity of transfection-grade plasmid DNA from bacterial cultures

Plasmid DNA of three different bacterial strains, transformed with plasmids containing either a 1482 bp, a 982 bp or a 359 bp insert, 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 Transfection-grade kit on the ep*Motion*® 5075vt. Total purity was determined by UV spectrometry (A_{260}/A_{280}: dark blue bars; A_{260}/A_{230}: orange squares). All measured endotoxin contents showed significant less than 50 EU/µg DNA (EU = endotoxin units; data not shown).



Reproducible yields of plasmid DNA

Plasmid DNA of three different bacterial strains, transformed with plasmids containing either a 1482 bp, a 982 bp or a 359 bp insert, was isolated from 1.5 mL of bacterial cultures (*E. coli* DH 5α, high-copy plasmid pGEM®-T Easy). The reproducibility and integrity was analyzed by gel electrophoresis (10 µL per eluate; 1 % TAE gel; Marker (L): GeneRuler™ 1 kb DNA Ladder – Thermo Scientific).

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 kit on the ep*Motion*® 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 50 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 kit on the ep*Motion*® 5075vt.

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
ep*Motion*® 5075vt	Basic device incl. vacuum system, gripper, vac frame 2, vac frame holder, Eppendorf ThermoMixer®, epBlue™ software, mouse, waste box, 100–240 V ±10 % / 50–60 Hz ±5 %, 0.2 µL–1 mL	1	5075000304