NucleoMag® Plasmid

Purification of plasmid DNA with transfection-grade purity using the platform *epMotion*® 5075t

Application benefits

Experience rapid plasmid purification in proven 96-well format with the NucleoMag® Plasmid kit and epMotion®, featuring:

- Verified methods ensuring an automated purification process.
- Consistent recovery of plasmid DNA with reliable reproducibility in both yield and purity.
- High throughput capability, processing up to 96 samples in parallel.
- Flexible protocol for both options of lysate clarification

Keywords

Plasmid DNA, Nucleic acid extraction, Automated DNA purification, cloning, gene therapy, pharmaceutical research, protein production, gene editing, magnetic beads, Eppendorf, epMotion®, laboratory protocol optimization

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 magnetic bead based kit, NucleoMag® Plasmid, for the isolation of endotoxin reduced plasmid DNA. The kit combines automated 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).

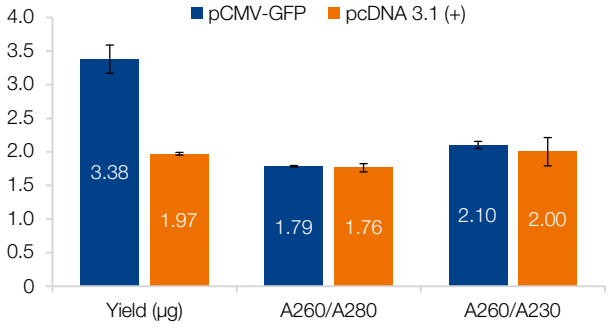
This application note describes the automated process on the liquid handling workstation epMotion® 5075t using the NucleoMag® Plasmid kit from MACHEREY-NAGEL. The novel optimized protocol allows the processing of a variable sample number in multiples of 8, depending on protocol choice.

NucleoMag® Plasmid	
Technology	Magnetic bead technology
Sample material	≤ 5 mL bacterial *E. coli* culture
Format	Variable sample number in multiples of 8 (8–96)
Typical yield	1–50 µg
Typical endotoxin levels	≤ 50 EU/µg
Elution volume	50–200 µL

Material and Methods

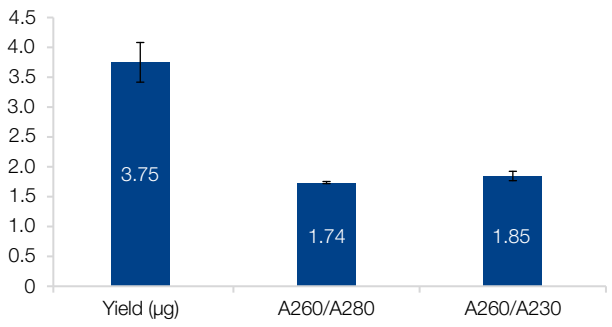
The optimized protocol is programmed to process up to 96 samples in parallel depending on mode of lysate clearance (Variable sample number in multiples of 8) and developed for the epMotion® 5075t platform. Cultivation and harvesting of bacterial cells is recommended to perform according to the NucleoMag® Plasmid user manual. All subsequent steps are performed on the epMotion® 5075t. 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 S3. The crude lysate can be cleared either by centrifugation or using NucleoMag® Clearing Beads, specialized paramagnetic beads for lysate clearing. For binding of nucleic acids to the paramagnetic beads, Binding Buffer PAB and the NucleoMag® M-Beads are added to the cleared lysate. After magnetic separation, endotoxins and proteins are removed by the patented Detoxification Buffer ERB. Further contaminations such as salts or residual ethanol are removed with Wash Buffer AQ and air drying. 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 two different bacterial strains, transformed with plasmids was isolated from 1 mL of bacterial cultures (*E. coli* TOP10, high-copy plasmid pCMV-GFP (dark blue bars) and pcDNA™ 3.1 (+) (orange bars) (n = 8) using the NucleoMag® Plasmid kit with lysate clearance via centrifugation on the epMotion® 5075t. Total yield and purity was determined by UV spectrometry. All measured endotoxin contents showed significantly less than 50 EU/µg DNA (EU = endotoxin units).



Isolation of plasmid DNA using NucleoMag® Clearing Beads for lysate clarification

Plasmid DNA isolated from 0.5 mL of bacterial cultures (*E. coli* TOP10, high-copy pCDNA 3.1 (+); n = 8) using the NucleoMag® Plasmid kit with lysate clearance via the NucleoMag® clearing bead technology on the epMotion® 5075t. Total yield and purity was determined by UV spectrometry. All measured endotoxin contents showed significantly less than 50 EU/µg DNA (EU = endotoxin units).

Lysate clearance	Plasmid	EU/µg DNA
Centrifugation	pCMV-GFP	7.30 ± 6.41
Centrifugation	pcDNA™ 3.1 (+)	10.37 ± 3.99
NucleoMag® Clearing Beads	pcDNA™ 3.1 (+)	27.85 ± 8.12

Low endotoxin levels using NucleoMag® Plasmid on liquid handling platforms

Plasmid DNA was isolated according to the user manual. Endotoxin units (EU) were determined by quantitative chromogenic LAL-test (n = 3)

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 NucleoMag® Plasmid kit on the epMotion® 5075t to speed up your nucleic acid purification workflow.

- Endotoxin removal wash buffer and optimized magnetic beads for highly pure plasmid DNA with less than 50 endotoxin units per µg DNA.
- Reliable performance and excellent yields using NucleoMag® Plasmid kit on the epMotion® 5075t.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® Plasmid	Kit based on magnetic bead technology for the isolation of transfection-grade plasmid DNA from bacterial cultures	1 x 96 / 4 x 96	744750.1 / .4
NucleoMag® SEP	Static magnetic separator	1	744900
NucleoMag® Clearing Beads	Lysate clarification based on magnetic bead technology during plasmid DNA isolation with the NucleoMag® Plasmid kit	1 x 96	744751.1
epMotion® 5075t	Basic device incl. Eppendorf ThermoMixer®, epBlue™ software, mouse, waste box, 100–240 V ± 10 % / 50–60 Hz ± 5 %, 0.2 µL–1 mL, EU-plug	1	5075000042*

*For more detailed information, please visit www.eppendorf.com. To contact Eppendorf SE, please email to eppendorf@eppendorf.com or visit www.eppendorf.com/contact.