

MACHEREY-NAGEL Application Note 06/2024 · Bioanalysis

NucleoSpin[®] 96 Plasmid

Automated mini preparation of plasmid DNA on the flowbot® ONE workstation

Application benefits

Experience rapid plasmid purification in proven 96-well format with the NucleoSpin[®] 96 Plasmid kit and flowbot[®] ONE, featuring:

- Pre-written and tested methods ensuring a semi 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.
- Accessible protocols through MACHEREY-NAGEL's technical automation support at automation-bio@mn-net.com.

Keywords

Plasmid DNA, Nucleic acid extraction, Automated DNA purification, cloning, gene therapy, pharmaceutical research, protein production, gene editing, vacuum, Flowbot ONE, Flowrobotics automation system, laboratory protocol optimization



Flow Robotics flowbot® ONE

The flowbot® ONE Workstation was equipped with the NucleoVac 96 vacuum manifold an external vacuum pump and digital vacuum regulator

Introduction

Plasmid extractions play a pivotal role in biological research and biotechnology, serving as a cornerstone for various applications. The extraction of plasmids from bacterial hosts is a fundamental technique facilitating the manipulation, analysis, and utilization of genetic material, offering researchers and biotechnologists a versatile toolset to explore and harness the intricacies of DNA. In this context, plasmid extraction emerges as an indispensable procedure, empowering scientists to delve into the realms of genetic engineering, protein production, pharmaceutical and medical advancements such as gene therapy, and diagnostic technologies.

Automating this process offers significant advantages in terms of 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. Moreover, automation enhances reproducibility and standardization, ensuring that each extraction is performed with precision and consistency. Therefore, the automation of plasmid extraction represents a valuable advancement in biological research and biotechnology, facilitating faster, more reliable, and standardized processes for a wide range of applications.

| NucleoSpin [®] 96 Plasmid | |
|------------------------------------|---|
| Technology | Silica membrane |
| Processing | Manual or automated |
| Sample material | Bacteria, E. coli |
| Target molecules | plasmid DNA |
| Vector size | < 25 kbp |
| Sample numbers on flowbot® ONE | 96 samples with 1000 µL 8-channel pipette configuration |

| flowbot [®] ONE | |
|--------------------------|---|
| Technology | Automated liquid handling platform equipped with electronic pipettes |
| Sample numbers | 1–96 samples |
| Deck positions | Configurable platform with 12 deck slots + tip waste |
| Pipetting volume | 2 pipette modules (choose between 1, 4 and 8-channel) |
| | Volume ranges: $1-20 \ \mu\text{L}$, $2-200 \ \mu\text{L}$ and $10-1000 \ \mu\text{L}$ |

Material and Methods

The extraction of plasmid DNA was carried out utilizing MACHEREY-NAGEL's NucleoSpin® 96 Plasmid kit on the Flow Robotics flowbot® ONE platform. The verified protocol gives the researcher an easy start to automatically perform 8–96 plasmid purifications per run. The sample resuspension with buffer A1 of bacterial pellets harvested from up to 5 mL culture, alkaline lysis with buffer A2, neutralization reaction with buffer A3 followed and sample transfer is completely automated. Following this, the crude lysate underwent vacuum filtration on the integrated NucleoVac 96 manifold through the NucleoSpin® 96 Filter Plate. After brief reassembly of the vacuum manifold the binding of plasmid DNA to the silica membrane and removing of contaminants such as salts, proteins, or endotoxins through three consecutive washing steps take place. The highly purified plasmid DNA was ultimately eluted under low ionic strength conditions using a slightly alkaline elution buffer. All pipetting and Vacuum filtration steps were carried out on the flowbot® ONE.

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Application Data

DNA extraction was conducted from four different plasmid constructs and bacterial strains under different growth conditions. *E. coli* Top10 (pCMW-GFP) was cultivated in 1 mL cultures grown in 96-deep-well culture plates while *E. coli* Top10 (pcDNA), *E. coli* DH5a (pGEM) and *E. coli* Stbl3 (pUC19) were cultivated in high density growth conditions in circle grow media. The automated NucleoSpin[®] 96 Plasmid kit was performed by the flowbot[®] ONE system. DNA quantity (top, blue bars) and purity was determined photometrically Ratio A260/A280 (bottom, blue bars) and Ratio A260/A230 (bottom, orange boxes). Results show high purity with little deviation and expected yield depending of the growth conditions of those cultures.



Eight replicates of two different plasmid DNA isolated on the flowbot® ONE

Consistent DNA quality and integrity was visualized via gel electrophoresis (1% TAE-gel), revealing a majority of supercoiled plasmid DNA. On the top (A) eight replicates of *E. coli* Top10 (pcDNA) grown under high density conditions are shown. The lower part (B) displays eight replicates extracted from *E. coli* Top10 (pCMV-GFP) grown over night in a 96-deep-well culture plate. Both gel pictures feature the ThermoScientific GeneRuler 1kb DNA Ladder as a marker on the left. These results demonstrate the efficacy of the NucleoSpin[®] 96 Plasmid kit and the flowbot[®] ONE system in achieving reliable and high-quality DNA isolations.

Ordering information

| Product | Specifications | Pack of | REF |
|---|--|---|---------------------------------|
| NucleoSpin [®] 96 Plasmid | Rapid isolation of plasmid DNA in proven 96-well format; including NucleoSpin [®] 96 Plasmid Filter & Binding Plate, buffers and plastic consumables | 1 × 96 preps 4 × 96 preps 24 × 96 preps | 740625 740625.4 740625.24 |
| Square-well Block | 96 Deep-well Plate to provide cleared lysates | 4 × pieces 24 × pieces | 740481 740481.24 |
| Flow Robotics flowbot® ONE Workstation | Automated platform for automated liquid handling and sample preparation | 1–96 samples | |

NucleoSpin[®] is a registered trademark of MACHEREY-NAGEL (contact: automation-bio@mn-net.com); Flowbot[®] is a registered trademark of FlowRobotics; Applied Biosystems[®] is a registered trademark of Applied Biosystems.

*For more detailed information, please visit www.flow-robotics.com. To contact FlowRobotics Sales or to schedule a demo, please email info@flow-robotics.com.