NucleoBond® RS Columns

Preparative scale isolation of vaccine-grade plasmid DNA using the MEDNA Scientific VASO System

Application benefits

MACHEREY-NAGEL introduces a novel application note, elucidating the seamless integration of NucleoBond® RS columns with the VASO System by MEDNA Scientific:

- Vaccine-grade plasmid DNA with endotoxin levels ≤ 0.01 EU/µg DNA
- Scalable system for up to 200 mg
- Automated VASO fluid handling system
- Utilizes MEDNA's ICON control system for precise operation and real-time monitoring
- High-quality plasmids as starting material for applications, like lentivirus/AAV production or IVT-RNA (e.g. mRNA)

Keywords

Large scale plasmid purification, endotoxin-free, vaccine-grade, FPLC processing, scalable system, lentivirus / AAV production, IVT-RNA, mRNA, MEDNA Scientific, VASO system, MACHEREY-NAGEL

Introduction

The purification of vaccine-grade plasmid DNA holds paramount importance in the production of advanced therapeutics, such as mRNA-based vaccines and gene therapy treatments, where the quality of the starting material directly impacts the efficacy and safety of the final product. MACHEREY-NAGEL introduces the NucleoBond® RS columns, designed for preparative-scale isolation of vaccine-grade plasmid DNA with endotoxin levels ≤ 0.01 EU/µg DNA. In collaboration with MEDNA Scientific, this application note explores the integration of NucleoBond® RS columns with the VASO System—an automated fluid handling system featuring MEDNA's ICON control system. The VASO System incorporates in-line sensors, including disposable UV detectors, enabling real-time monitoring of critical parameters such as UV absorbance at specific wavelengths (e.g., 260 nm and 280 nm). With its capability for automated procedures and connectivity to cloud servers, the VASO System offers researchers enhanced flexibility and efficiency in plasmid DNA purification. This collaborative effort underscores a significant advancement in preparative-scale plasmid DNA isolation, promising improved quality and throughput in bioprocessing workflows.

	NucleoBond® RS 10	NucleoBond® RS 50	NucleoBond® RS 100	NucleoBond® RS 200
REF	743502	743503	743504	743505
Theoretical binding capacity	Up to 10 mg	Up to 50 mg	Up to 100 mg	Up to 200 mg
ODV_{max}	3.000	15.000	30.000	54.000
Cell wet $weight_{max}$	8 g	40 g	80 g	145 g
Exemplary technical data (average plasmid DNA qualities)				
Endotoxins (EU/µg DNA)	0.009 ± 0.004	0.007 ± 0.006	0.003 ± 0.002	0.003 ± 0.003
A260 / A280	1.99 ± 0.04	1.97 ± 0.02	1.97 ± 0.04	1.98 ± 0.01
Supercoiled form (ccc) in %	92.3 ± 3.0	93.8 ± 2.1	93.0 ± 3.1	95.2 ± 2.1

Parameter	Method	Criterion
Integrity	AGE / Fragment Analyzer	≥ 90% supercoiled form
Purity	UV spectrum	A260/A280 1.8 – 2.0 A260/A230 1.9 – 2.3
Endotoxin levels	LAL / rFC	≤ 0.01 EU/µg DNA
Host DNA	qPCR	≤ 1%
Host RNA	AGE Fluorometric Quantification	Not visible at 200 ng DNA ≤ 2%
Host Protein	BCA	≤ 1%

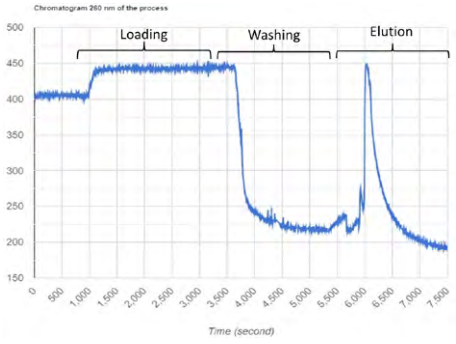
MEDNA VASO Q1801 (Gen 3) Systems	
Technology	VASO Fluid Handling System
Product details	https://mednabio.com/product-detail/P-247
Applications	Scalable Processes and Operations Production scale, high throughput full automation protein purification and nucleic acid purification Research scale full automation protein purification and nucleic acid purification Supports Size Exclusion Chromatography Configurations Supports Ion Exchange Chromatography Configurations Supports Affinity Chromatography Configurations Integration of external devices into automated process Advanced automation capabilities that are highly adaptable with a user-centered design
Contact information	Website: https://mednabio.com Email: support@mednabio.com Phone: 469-250-4424

Material and Methods

The extraction of plasmid DNA was carried out utilizing MACHEREY-NAGEL´s NucleoBond® RS 10 column on the MEDNA Scientific VASO System. Up to 8 g of bacterial pellet was resuspended in Buffer RES-EF, supplemented with RNase A. Afterwards sample was carefully lysed with Buffer LYS-EF and then neutralized by using NEU-EF, resulting in precipitation of proteins, chromosomal DNA and other cellular debris. For clarification of the crude lysate NucleoBond® Bottle Top Filter Type 2 was used to ensure high plasmid quality and fast column flow rates. After clarification, the lysate was loaded onto the NucleoBond® RS 10 column with a flowrate of 5 mL/min. After binding of the plasmid DNA to the column resin, subsequent washing steps with Buffer ENTO-EF and Buffer WASH-EF were carried out to efficiently remove contaminants and endotoxins. Elution of the high-quality plasmid DNA was performed under high salt conditions using ELU-EF, followed by plasmid DNA precipitation with room temperature isopropanol, ethanol (70 % EtOH) washing and reconstitution with TE-EF.

Application Data

A



Chromatogram (260 nm) of a NucleoBond® RS 10 plasmid purification

Icon Absorbance Chromatogram obtained upon automated plasmid purification using the NucleoBond® RS 10 column. A precise peak at 260 nm can be detected during the elution step, indicating the distinct elution of plasmid DNA from the column. The plasmid DNA was isolated from approx. 1 L of bacterial culture. A total of 8.3 mg pure plasmid DNA was isolated with a purity ratio A260/A280 of 1.84.

B

Overview of the VASO – NucleoBond RS workflow

The VASO – NucleoBond® RS combination offers an automated workflow for the processing of large-scale plasmid DNA purification. The buffers and the NucleoBond® RS columns are connected to the VASO system. Prior to the purification process, sample preparation (e.g., lysis and neutralization of the culture) is performed. The subsequent purification process is controlled via the intuitive MEDNA ICON software, requiring a minimum of hands-on time.

Ordering information

To ensure maximum flexibility for our customers, we offer all components needed for plasmid purification with our NucleoBond® RS columns as standalone products. For more detailed information regarding equipment and quantities of individual components required per prep please check the user manual.

Product	Pack of / volume	REF
NucleoBond® RS 10	5 columns	743502
NucleoBond® RS 50	1 column	743503
NucleoBond® RS 100	1 column	743504
NucleoBond® RS 200	1 column	743505
RES-EF	1000 mL / 3000 mL	740386.1000 / .3000
LYS-EF	1000 mL / 3000 mL	740387.1000 / .3000
NEU-EF	1000 mL / 3000 mL	740388.1000 / .3000
EQ-EF	1000 mL / 3000 mL	740380.1000 / .3000
ENTO-EF	1000 mL / 3000 mL	740381.1000 / .3000
WASH-EF	1000 mL / 3000 mL	740392.1000 / .3000
ELU-EF	900 mL / 3000 mL	740393.900 / .3000
TE-EF	1000 mL	740797.1
H2O-EF (nuclease free)	1000 mL	740798.1
Liquid RNase A	2.5 mL	740397
VASO	MEDNA Bioscience VASO system	Q1600