

MACHEREY-NAGEL

# User manual



## Plasmid DNA concentration and desalination


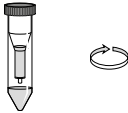

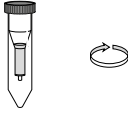
- NucleoSpin® Finisher Midi

November 2023 / Rev. 02

# Plasmid DNA concentration and desalination

## Protocol at a glance (Rev. 02)

### NucleoSpin® Finisher Midi

<b>1 Adjust precipitation conditions</b>		2.5 mL Buffer FB to 5 mL anion exchange eluate  Vortex for 5 s
<b>2 Filtrate</b>		Load mixture  1 min, 3,000 x g  Discard flowthrough
<b>3 Wash and dry filter membrane</b>		2 mL Buffer A4  2 min, ≥ 3,000 x g
<b>4 Elute DNA</b>		200 – 500 µL H <sub>2</sub> O-EF  RT, 1 min  2 min, ≥ 3,000 x g

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## Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents and equipment to be supplied by user	4
1.3	About this user Manual	4
2	Product description	5
2.1	Basic principle	5
2.2	Kit specifications	5
2.3	Recommendations for pipetting of Buffer FB	5
2.4	Elution procedures	6
3	Storage conditions and preparation of working solutions	7
4	Safety instructions	7
4.1	Disposal	7
5	Protocol for plasmid concentration and desalination	8
5.1	Plasmid purification from anion exchange prep eluates	8
5.2	DNA precipitation from maxi prep eluates	9
6	Appendix	9
6.1	Troubleshooting	9
6.2	Ordering information	10
6.3	Product use restriction / warranty	11

# 1 Components

## 1.1 Kit contents

REF	NucleoSpin® Finisher Midi	
	10 preps 740439.10	50 preps 740439.50
Buffer FB	30 mL	150 mL
Buffer A4 (Concentrate)*	6 mL	25 mL
H <sub>2</sub> O-EF	13 mL	30 mL
NucleoSpin® Finisher Midi Columns (in 50 mL Collection Tubes)	10	50
User manual	1	1

## 1.2 Reagents and equipment to be supplied by user

### Reagents

- 96–100 % ethanol

### Equipment

- Centrifuge capable of reaching  $\geq 3,000 \times g$  with adaptors for 50 mL centrifuge tubes
- Pipettes and pipette tips for 0.1–1 mL and 0.5–10 mL

## 1.3 About this user Manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at [www.mn-net.com](http://www.mn-net.com).

Please contact Technical Service regarding information about changes of the current user manual compared to previous or updated revisions.

\* For preparation of working solution and storage conditions see section 3.

## 2 Product description

### 2.1 Basic principle

**NucleoSpin® Finisher** kits supersede the commonly used and time consuming isopropanol precipitation of DNA from anion exchange eluates by allowing the rapid DNA precipitation onto a filter membrane, followed by a combined washing / drying step and a convenient elution with a variable elution volume. All steps are conveniently performed in a centrifuge which allows the parallel purification of multiple samples.

DNA is precipitated by Buffer FB and filtered by the specially designed matrix of the novel **NucleoSpin® Finisher Columns**, followed by a combined washing and drying step with ethanolic Wash Buffer A4 to remove salts, impurities, and ethanol.

Afterwards, plasmid DNA can be eluted in supplied endotoxin-free H<sub>2</sub>O-EF. The applied elution volume can be adjusted from 100 µL to 1.5 mL according to the expected amount of plasmid DNA to ensure optimal yield and concentration (see section 2.4 for recommendations concerning the optimal elution volume).

### 2.2 Kit specifications

The **NucleoSpin® Finisher Midi** kit is specifically designed for the fast and parallel purification and concentration of up to 2 mg of plasmid DNA from anion exchange preparation eluates. The **NucleoSpin® Finisher Midi** kit can be used in combination with the **NucleoBond® Xtra Midi** and the **NucleoBond® PC 100** kits.

For research use only.

Purification of DNA from maxi prep eluates (**NucleoBond® Xtra Maxi** and **NucleoBond® PC 500**) is also possible, but will require multiple loading steps and additional precipitation buffer (to be ordered separately).

### 2.3 Recommendations for pipetting of Buffer FB

**!** Buffer FB is viscous. Use of **reverse pipetting** is recommended to ensure accurate volumes. Reverse pipetting is done by pressing down the pipette's plunger button all the way down to the second stop before slowly aspirating the Buffer FB until the plunger button rests again in the starting position. The buffer volume inside the pipette tip is larger than set now, so when dispensing the Buffer FB to the anion exchange eluates, be sure to dispense to the first stop only! Liquid remaining in the pipette tip can be dispensed back to the original solution.

For further details concerning the reverse pipetting technique and liquid handling of viscous fluids, you may also check your pipette manufacturer's information material.

## 2.4 Elution procedures

Total yield depends on the final DNA concentration in the eluates.

A higher concentration than 2 µg/µL of DNA is highly viscous and therefore difficult to elute from a spin column. As a result, DNA will not elute completely if the elution buffer volume is too low.

To prevent reduced total yield as side-effect of high concentration, it is recommended to measure the DNA content of the anion exchange eluate and to choose the total elution volume accordingly to gain a final concentration of 1–2 µg/µL.

- For a high **concentration**, use an elution volume of 200 µL, reload the eluate onto the column, and repeat the elution step.
- For a high **yield**, use an elution volume of 500 µL, reload the eluate onto the column, and repeat the elution step.

In general, it is advantageous to incubate the Elution Buffer on the membrane at room temperature or elevated temperatures (e.g., 50–70 °C) for 1–5 min.

### Recommended elution volumes according to expected yield

Kit	DNA yield	Recommended elution volume
NucleoBond® PC 100	up to 100 µg	100–150 µL
NucleoBond® Xtra Midi	up to 500 µg	200–500 µL
NucleoBond® PC 500	up to 500 µg	200–500 µL
NucleoBond® Xtra Maxi	up to 1500 µg	2 × 500 µL

### 3 Storage conditions and preparation of working solutions

All kit components can be stored at room temperature (18–25 °C) and are stable for at least two years.

Before starting any **NucleoSpin® Finisher Midi** protocol, prepare the following:

- **Wash Buffer A4:** Add the given volume of ethanol (96–100 %) as indicated on the bottle or in the table below to **Buffer A4 Concentrate** before first use. Mark the label on the bottle to indicate that the ethanol is added. Prepared Buffer A4 is stable at room temperature (18–25 °C) for at least one year.

NucleoSpin® Finisher Midi		
REF	10 preps 740439.10	50 preps 740439.50
Buffer A4 (Concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol

### 4 Safety instructions

When working with the **NucleoSpin® Finisher Midi** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at <http://www.mn-net.com/msds>).



The waste generated with the **NucleoSpin® Finisher Midi** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

#### 4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

## 5 Protocol for plasmid concentration and desalination

### 5.1 Plasmid purification from anion exchange prep eluates

Before starting the preparation:

Check if Buffer A4 was prepared according to section 3.

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#### 1 Adjust precipitation conditions

Add **2.5 mL Buffer FB** to **5 mL** anion exchange eluate.

*Reverse pipetting is recommended (see section 2.3).*

**Vortex for 5 s.**

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#### 2 Filtrate

**Load the mixture** onto a **NucleoSpin® Finisher Midi Column** combined with a 50 mL Collection Tube.

Centrifuge for **1 min** at **3,000 x g**. If the filtration is not complete, repeat centrifugation until all fluid has passed the filter membrane.

Discard flowthrough and place the NucleoSpin® Finisher Midi Column back into the empty Collection Tube.

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#### 3 Wash and dry filter membrane

Apply **2 mL Buffer A4** onto the column. Centrifuge for **2 min** at **≥ 3,000 x g**. Discard collection tube and place the NucleoSpin® Finisher Midi Column into a new 50 mL Collection Tube (not supplied).

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#### 4 Elute DNA

Add **200–500 µL** (minimum 100 µL, maximum 1.5 mL) of **nuclease- and endotoxin-free H<sub>2</sub>O-EF** onto the membrane and incubate at **room temperature** for **1 min**.

Centrifuge for **2 min** at **≥ 3,000 x g**.

*Optional: Repeat elution with the eluate as elution buffer for optimal recovery. See section 2.4 for further recommendations.*

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## 5.2 DNA precipitation from maxi prep eluates

*Note:* The supplied volume of Buffer FB in this kit will not be sufficient for purification of maxi prep eluates. Additional buffer has to be ordered separately (see ordering information).

Add **7.5 mL Buffer FB** to **15 mL** anion exchange maxi prep eluate. Load up to 8 mL of the mixture onto a **NucleoSpin® Finisher Midi Column** combined with a 50 mL Collection Tube. Centrifuge for **1 min** at **3,000 x g**. If the filtration is not complete, repeat centrifugation until all fluid has passed the filter membrane.

Discard flowthrough and place the NucleoSpin® Finisher Midi Column back into the empty collection tube. Repeat this step until all the mixture from step 1 has passed the filter membrane.

Continue with step 3 of the standard protocol.

## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
	<p><i>No plasmid DNA present in anion exchange eluates.</i></p> <ul style="list-style-type: none"> <li>• Measure DNA yield after anion exchange prep.</li> </ul>
No or low DNA yield	<p><i>Insufficient amount of Buffer FB added.</i></p> <ul style="list-style-type: none"> <li>• Buffer FB is viscous, make sure to add the correct volume.</li> <li>• Use “reverse pipetting” to avoid inaccurate pipetting of precipitation buffer (see section 2.3).</li> <li>• Precipitation works best when 0.5 vol of Buffer FB are added to each vol of anion exchange eluate. When using other volumes than those of the standard procedure, adjust volume of Buffer FB accordingly.</li> </ul>

## 6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Finisher Midi	740439.10	10 preps
	740439.50	50 preps
MN Vacuum Manifold	740299	1
NucleoSnap® Finisher Midi	740434.10	10 preps
	740434.50	50 preps
NucleoSnap® Finisher Maxi	740435.10	10 preps
	740435.50	50 preps
Buffer FB	740438.1000	1000 mL
Buffer A4 (Concentrate)	740914.1	200 mL
H <sub>2</sub> O-EF	740798.1	1000 mL

### 6.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the customer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

Last updated: 08/2022, Rev. 04

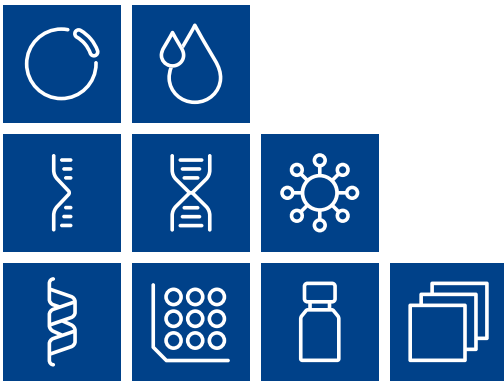
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