Plasmid DNA concentration and desalination

User manual

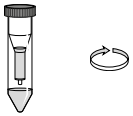
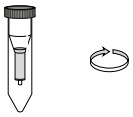
NucleoSpin® Finisher Midi

February 2016 / Rev. 01

MACHEREY-NAGEL
www.mn-net.com

Plasmid DNA concentration and desalination

Protocol-at-a-glance (Rev. 01)

		NucleoSpin® Finisher Midi
1 Adjust precipitation conditions		2.5 mL Buffer FB to 5 mL anion exchange eluate Vortex for 5 s
2 Filtrate		Load mixture 1 min, 3,000 x *g* Discard flow-through
3 Wash and dry filter membrane		2 mL Buffer A4 2 min, ≥ 3,000 x *g*
4 Elute DNA		200–500 µL H_2O-EF RT, 1 min 2 min, ≥ 3,000 x *g*

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-270 · Fax: +49 24 21 969-199 · tech-bio@mn-net.com · ***www.mn-net.com***

Table of contents

1 Components

1.1 Kit contents

	NucleoSpin® Finisher Midi	
REF	**10 preps 740439.10**	**50 preps 740439.50**
Buffer FB	30 mL	150 mL
Buffer A4 (Concentrate)*	6 mL	25 mL
H_2O-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 ≥ 3,000 x *g* with adaptors for 50 mL centrifuge tubes
- Pipettes and pipette tips for 0.1–1 mL and 0.5–10 mL

* 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_2O-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.

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 x 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

The NucleoSpin® Finisher Midi kit does not contain hazardous components.

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.

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.**

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 flow-through and place the NucleoSpin® Finisher Midi Column back into the empty Collection Tube.

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).

4 Elute DNA

Add **200–500 µL** (minimum 100 µL, maximum 1.5 mL) of **nuclease- and endotoxin-free H_2O-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.

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 flow-through 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**
No or low DNA yield	*No plasmid DNA present in anion exchange eluates.* • Measure DNA yield after anion exchange prep.
	Insufficient amount of Buffer FB added. • Buffer FB is viscous, make sure to add the correct volume. • Use “reverse pipetting” to avoid inaccurate pipetting of precipitation buffer (see section 2.3). • 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.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Finisher Midi	740439.10 740439.50	10 preps 50 preps
MN Vacuum Manifold	740299	1
NucleoSnap Finisher Midi	740434.10 740434.50	10 preps 50 preps
NucleoSnap Finisher Maxi	740435.10 740435.50	10 preps 50 preps
Buffer FB	740438.1000	1000 mL
Buffer A4 (Concentrate)	740914.1	200 mL
H_2O-EF	740798.1	1000 mL

6.3 Product use restriction / warranty

NucleoSpin® Finisher Midi kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

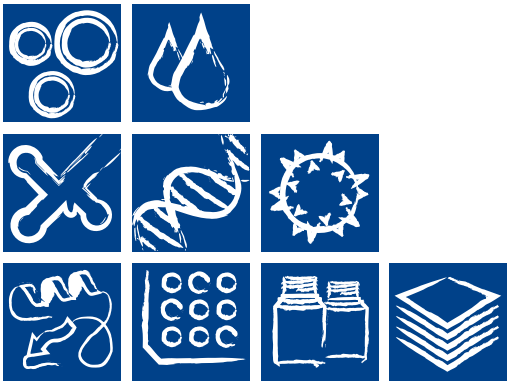
The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent 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 the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications

mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07/2010, Rev. 03

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 (0) 24 21 969 270
e-mail: tech-bio@mn-net.com



www.mn-net.com

MACHEREY-NAGEL

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

DE / International:
Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:
Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

A054032/0560.5