Plasmid DNA concentration and desalination

User manual

NucleoSnap® Finisher Midi
NucleoSnap® Finisher Maxi

October 2017 / Rev. 02

MACHEREY-NAGEL
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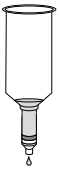
Plasmid DNA concentration and desalination

Protocol at a glance (Rev. 01)

	Midi NucleoSnap® Finisher Midi		Maxi NucleoSnap® Finisher Maxi
1 Adjust binding conditions	2.5 mL Buffer FB Mix		7.5 mL Buffer FB Mix
2 Filtrate precipitate	Connect NucleoSnap® Column to vacuum Load sample -0.3 bar*		Connect NucleoSnap® Column to vacuum Load sample -0.3 bar*
3 Wash precipitate	2 mL Buffer A4 -0.3 bar*		4 mL Buffer A4 -0.3 bar*
4 Separate column	Remove upper part and discard		Remove upper part and discard
5 Dry silica membrane	11,000 x *g*, 1 min		11,000 x *g*, 1 min
6 Elute DNA	200 µL H_2O-EF 11,000 x *g*, 1 min		500 µL H_2O-EF 11,000 x *g*, 1 min

*Reduction of atmospheric pressure. Use of a vacuum regulator (e.g., NucleoVac Vacuum Regulator) to prevent a reduced recovery due to higher vacuum forces is highly recommended, see ordering information.

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***

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1 Components

1.1 Kit contents

	NucleoSnap® Finisher Midi		NucleoSnap® Finisher Maxi	
REF	**10 preps 740434.10**	**50 preps 740434.50**	**10 preps 740435.10**	**50 preps 740435.50**
Buffer FB	30 mL	150 mL	90 mL	400 mL
Buffer A4 (Concentrate)	6 mL	25 mL	2 x 6 mL	2 x 25 mL
H_2O-EF	13 mL	30 mL	30 mL	60 mL
NucleoSnap® Finisher Columns	10	50	10	50
Collection Tubes	10	50	10	50
User manual	1	1	1	1

1.2 Reagents and equipment to be supplied by user

Reagents

- 96–100 % ethanol

Equipment

- Vacuum manifold with Luer adapters (NucleoVac Mini Adapters)
- Vacuum pump capable of reaching -0.3 bar (9–10 in. Hg)
- Centrifugation tubes (2 mL)
- Centrifuge capable of reaching ≥ 10,000 x *g*
- Pipettes and pipette tips for 0.1–1 mL and 0.5–10 mL

2 Kit specifications

- The **NucleoSnap® Finisher** kits are specifically designed for the fast and parallel purification and concentration of up to 2 mg of plasmid DNA from anion exchange preparation eluates.
- The **NucleoSnap® Finisher Midi** kit can be used in combination with the NucleoBond® Xtra Midi and the NucleoBond® PC 100 kits.
- The **NucleoSnap® Finisher Maxi** kit can be used in combination with the NucleoBond® Xtra Maxi and the NucleoBond® PC 500 kits.
- NucleoBond® PC 2000 eluates require additional Buffer FB (see ordering information, section 6.2) and can be used according to the NucleoSnap® Finisher Maxi protocol. Filtration times will increase when more than 1.5 mg DNA are being purified.
- Purification of DNA from samples with low ionic strength like pure or slightly buffered water is possible according to protocol 5.2 but will require additional Buffer PL3 (see ordering information, section 6.2).

2.1 About NucleoSnap® Finisher kits

NucleoSnap® Finisher kits supersede the commonly used and time consuming isopropanol precipitation and centrifugation steps by allowing the rapid DNA precipitation and concentration of large volumes under vacuum.

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

To enable the use of a table top centrifuge, the **NucleoSnap® Finisher Column** is composed of an upper funnel component which can be removed from the lower Mini spin column part by a simple break action. Then the Mini spin column can be centrifuged in a standard table top centrifuge to remove residual ethanol.

Afterwards, plasmid DNA can be eluted in supplied endotoxin-free H_2O-EF or any other suitable elution solution like slightly alkaline TRIS solutions or TE buffer. 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 chapter 2.3 for recommendations concerning the optimal elution volume.

2.2 Setup of NucleoSnap® Finisher Columns

NucleoSnap® Finisher Columns are connected to a vacuum manifold either by a direct Luer connection or by the use of a NucleoVac Mini Adapter or NucleoVac Valves (see ordering information, section 6.2). A valve is useful to switch off vacuum selectively when using a large number of columns at the same time to prevent a pressure loss through empty columns and excessive filtration of potentially contaminated air.

It is highly recommended to use a vacuum regulator (e.g., NucleoVac Vacuum Regulator, see ordering information) between vacuum manifold and vacuum source. Higher vacuum forces than -300 mbar* will result in a reduced recovery of nucleic acids

The space between each two used inlets of the vacuum manifold should be sufficient not to bend or dislodge **NucleoSnap® Finisher Columns** attached to the vacuum manifold.

The **NucleoSnap® Finisher Columns** consist of one piece but can be divided into two parts: a lower Mini spin column part and an upper funnel part. Handle the columns carefully to prevent accidental damage to the predetermined breaking point!

! 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 buffer container.

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

2.3 Elution procedures

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

A higher concentration than 2 µg/µL of DNA is difficult to elute from a silica spin column. As a result, DNA will not elute completely if the elution buffer is already saturated with plasmid DNA.

To prevent reduced total yield as a 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.

- To increase **concentration**, perform 2 elution steps by reloading the eluate (generally recommended).
- To increase **yield**, perform 2 x 500 µL elution steps with fresh nuclease free H_2O-EF for a total elution volume of 1 mL. Measure DNA content in both fractions separately.

In general it is advantageous to incubate the elution buffer (e.g., supplied H_2O-EF, TE buffer or buffer of choice) on the membrane at room temperature or elevated temperatures (e.g., 50–70 °C) for 1–5 min.

*Reduction of atmospheric pressure.

Recommended elution volumes according to expected yield		
Kit	**DNA yield**	**Recommended volume for elution**
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
NucleoBond® PC 2000	up to 2000 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 **NucleoSnap® Finisher** 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.

	NucleoSnap® Finisher Midi		**NucleoSnap® Finisher Maxi**	
REF	**10 preps 740434.10**	**50 preps 740434.50**	**10 preps 740435.10**	**50 preps 740435.50**
Buffer A4 (Concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol	2 x 6 mL Add 24 mL ethanol to each bottle	2 x 25 mL Add 100 mL ethanol to each bottle

4 Safety instructions

The **NucleoSnap® Finisher** kits do not contain hazardous components.

Wear gloves and goggles and follow the manufacturers' safety instructions for using the vacuum manifold!

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.

All vacuum steps are performed with a pressure of about -0.3 bar* (10 in. Hg).

	Midi	Maxi
1	**Adjust precipitation conditions** Add **0.5 volumes** of **Buffer FB** to anion exchange eluate. *Reverse pipetting is recommended (see chapter 2.2).* **2.5 mL** / **7.5 mL** Vortex for 5 s.	
2	**Filtrate precipitate** Connect the **NucleoSnap® Finisher Column** to a vacuum manifold and load mixture from step 1 onto the column. Apply vacuum (-0.3 bar*) until the solution has completely passed and then turn vacuum off.	
3	**Wash precipitate** Add **Buffer A4** onto the column and apply vacuum (-0.3 bar*) until the solution has completely passed and then turn vacuum off. **2 mL** / **4 mL**	
4	**Separate column** Remove the **NucleoSnap® Finisher Column** from the vacuum manifold and place the bottom Mini spin column part into a 2 mL Collection Tube (supplied). Snap off the funnel part from the Mini spin column part (placed in the Collection Tube). Discard the funnel.	
5	**Dry silica membrane** Centrifuge Mini spin column and Collection Tube for **1 min** at **> 11,000 x *g*** to remove any residual ethanol. Discard the Collection Tube and place the Mini spin column in a new 2 mL Eution tube (not supplied).	

*Reduction of atmospheric pressure.

	Midi	Maxi

6 Elute DNA

Add **200–500 µL** of nuclease and endotoxin-free **H_2O-EF** onto the membrane (for recommended elution volumes, see section 2.3) and incubate at room temperature for 1 min.

Centrifuge for **1 min** at **> 10,000 x *g***.

Optional: Repeat elution with the eluate as elution buffer for optimal recovery. See chapter 2.3 for further hints.

Recommended for Maxi preps: Place Mini spin column in a new 2 mL elution tube (not supplied). Repeat elution with 500 µL of fresh H_2O-EF as elution buffer for optimal recovery. Check DNA concentration in both fractions.

Note: H_2O-EF can be replaced by TE buffer, slightly alkaline TRIS solution or any other suitable elution buffer.

5.2 Support Protocol for purification of DNA from samples with low ionic strength

Purification of DNA from samples with low ionic strength like DNA in water or slightly buffered solutions requires an adaption of buffer conditions, ionic strength, and pH value. Add 1/3 sample volume of Buffer PL3 (has to be ordered separately, see ordering information, section 6.2) to the sample prior to the addition of Buffer FB.

1 Adjust precipitation conditions

Add **0.33 volumes** of **Buffer PL3** to low ionic strength sample.

E.g., add 3 mL Buffer PL3 to 9 mL sample.

2 Precipitate DNA

Add **0.5 total volumes** of **Buffer FB** to the mixture.

E.g., add 6 mL Buffer FB to 12 mL mixture from step 1 (consisting of 9 mL sample and 3 mL Buffer PL3).

Reverse pipetting is recommended (see chapter 2.2).

Vortex for **5 s**.

Continue with step 2 of protocol 5.1

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.* • Precipitation Buffer FB is viscous, make sure to add the correct volume. • Use “reverse pipetting” to avoid inaccurate pipetting of precipitation buffer (see section 2.2) • 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. *Suboptimal pH of elution solution* • Optimal elution requires neutral to slightly alkaline conditions. When using other solutions than the supplied H_2O-EF, check pH and make sure the pH value is at least 7.0
Slow flow rates	*Excess plasmid input* • Plasmid DNA is filtrated on top of the filter membrane. Increasing amounts of plasmid DNA will lead to reduced flow rates when more than 1.5 mg DNA have been loaded. Loading DNA amounts higher than 2.5 mg might lead to column clogging. *Insufficient vacuum force* • The lower the vacuum force the slower the flow rate will be. Use vacuum pumps only that enable a minimum of -0.3 bar.

6.2 Ordering information

Product	REF	Pack of
NucleoSnap® Finisher Midi	740434.10 740434.50	10 preps 50 preps
NucleoSnap® Finisher Maxi	740435.10 740435.50	10 preps 50 preps
Vacuum manifold	740299	1
NucleoVac Vacuum Regulator	740641	1
NucleoVac Valves	740298.24	24
NucleoVac Mini Adapter	740297.100	100
Buffer FB	740438.1000	1000 mL
Wash Buffer A4 (Concentrate)	740914.1	200 mL
H_2O-EF	740798.1	1000 mL
Buffer PL3	740352.50 740352.1000	50 mL 1000 mL

6.3 Product use restriction / warranty

NucleoSnap® Finisher 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.

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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

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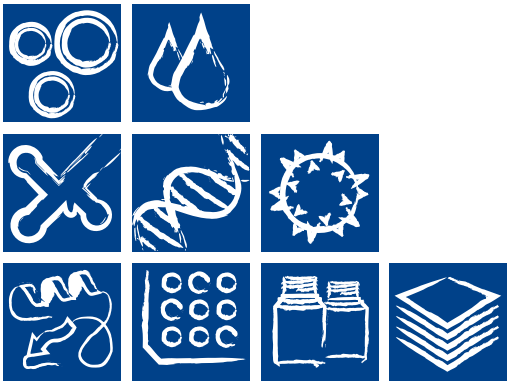

www.mn-net.com

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

A054031/1070.5