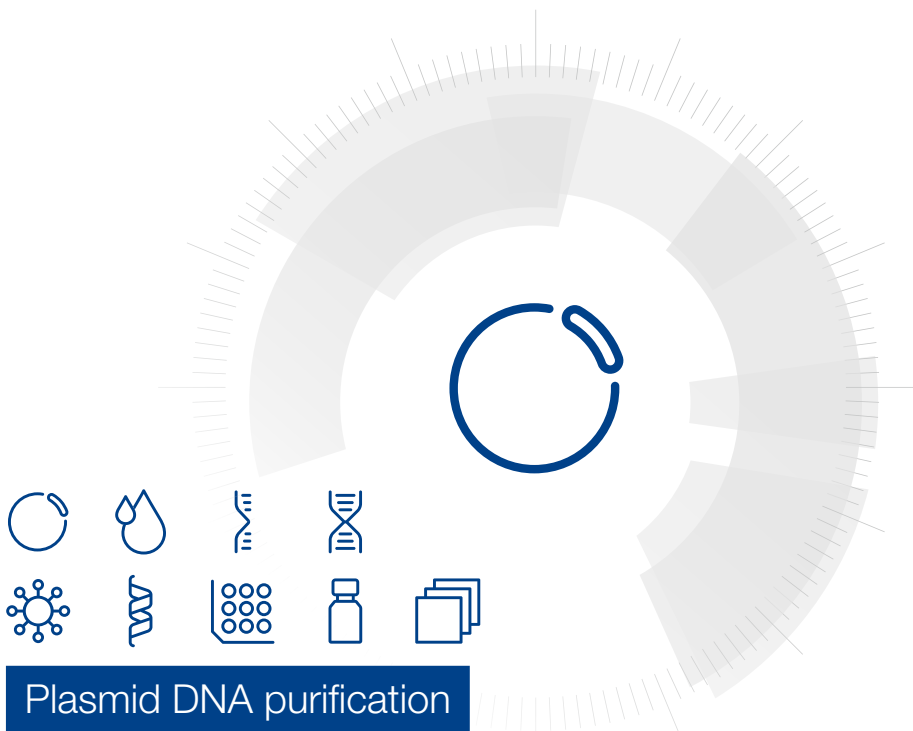


MACHEREY-NAGEL

# User manual



## Plasmid DNA purification

- NucleoSnap® Plasmid Midi

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## Contact MN

### Germany and international

MACHEREY-NAGEL GmbH & Co. KG  
Valenciener Str. 11 · 52355 Düren · Germany  
Tel.: +49 24 21 969-0  
Toll-free: 0800 26 16 000 (Germany only)  
E-mail: [info@mn-net.com](mailto:info@mn-net.com)

### Technical Support Bioanalysis

Tel.: +49 24 21 969-333  
E-mail: [support@mn-net.com](mailto:support@mn-net.com)

### USA

MACHEREY-NAGEL Inc.  
924 Marcon Blvd. · Suite 102 · Allentown PA, 18109 · USA  
Toll-free: 888 321 6224 (MACH)  
E-mail: [sales-us@mn-net.com](mailto:sales-us@mn-net.com)

### France

MACHEREY-NAGEL SAS  
1, rue Gutenberg – BP135 · 67720 Hoerdt Cedex · France  
Tel.: +33 388 68 22 68  
E-mail: [sales-fr@mn-net.com](mailto:sales-fr@mn-net.com)

MACHEREY-NAGEL SAS (Société par Actions Simplifiée) au capital de 186600 €  
Siret 379 859 531 00020 · RCS Strasbourg B379859531 · N° intracommunautaire FR04 379 859 531








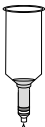




### Switzerland

MACHEREY-NAGEL AG  
Hirsackerstr. 7 · 4702 Oensingen · Switzerland  
Tel.: +41 62 388 55 00  
E-mail: [sales-ch@mn-net.com](mailto:sales-ch@mn-net.com)

# Plasmid DNA purification

## Protocol at a glance (Rev. 04)

### NucleoSnap® Plasmid Midi

1 Harvest bacterial cells		4,500–6,000 x <i>g</i> 4 °C, ≥ 10 min
2 Resuspend bacterial cells		5 mL SN1 Resuspend bacterial cells completely
3 Lyse cells		5 mL SN2 RT, max. 2 min
4 Neutralize		5 mL SN3 Mix thoroughly until colorless
5 Clarify lysate	 	Transfer lysate to NucleoSpin® Plasmid Filter Column 3,000 x <i>g</i> , 2 min Discard Plasmid Filter Column
6 Precipitate DNA		7 mL SN4 Mix
7 Filtrate DNA	 Vacuum	Load onto Snap Column -0.3 bar* (passage approx. 2–3 min)
8 Wash silica membrane	 Vacuum	<b>1<sup>st</sup> wash</b> 2 mL SN5 -0.3 bar* <b>2<sup>nd</sup> wash</b> 4 mL SN6 -0.3 bar*
9 Dry silica membrane	 	Remove upper column part and discard 10,000 x <i>g</i> , 1 min
10 Elute DNA	 	200-500 µL SNE 10,000 x <i>g</i> , 1 min

\* Reduction of atmospheric pressure

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# 1 Components

## 1.1 Kit contents

<b>NucleoSnap® Plasmid Midi</b>		
<b>REF</b>	<b>10 preps 740494.10</b>	<b>50 preps 740494.50</b>
Resuspension Buffer SN1	100 mL	2 × 150 mL
Lysis Buffer SN2	100 mL	2 × 150 mL
Neutralization Buffer SN3	100 mL	2 × 150 mL
Precipitation Buffer SN4	90 mL	400 mL
Endotoxin Removal Buffer SN5	25 mL	125 mL
Wash Buffer SN6 (Concentrate)*	12 mL	50 mL
Elution Buffer SNE**	13 mL	60 mL
RNase A (lyophilized)*	40 mg	2 × 60 mg
NucleoSpin® Plasmid Filter Columns	10	50
NucleoSnap® Plasmid Columns	10	50
Collection Tubes (2 mL)	10	50
User manual	1	1

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

\*\* Composition of Elution Buffer SNE: 5 mM Tris/HCl, pH 8.5.

## 1.2 Reagents and equipment to be supplied by user

### Reagents

- 96–100% ethanol

### Equipment

- Vacuum manifold with Luer adapters
- NucleoVac Vacuum Regulator (e.g., REF 740641)
- NucleoVac Valves (e.g., REF 740298.24)
- Vacuum pump capable of reaching -0.3 bar\* (~ 10 in. Hg)
- Centrifuge with swing-out rotor capable of reaching  $\geq 3,000 \times g$  for 50 mL tubes
- Microcentrifuge capable of reaching  $\geq 10,000 \times g$
- Centrifugation tubes (2 mL)
- Pipettes and pipette tips for 0.1–1 mL and 0.5–10 mL

## 1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSnap® Plasmid Midi** kit is used for the first time. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available online at [www.mn-net.com](http://www.mn-net.com). Please visit the MACHEREY-NAGEL website to verify that you are using the latest revision of this user manual.

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\* Reduction of atmospheric pressure

## 2 Product description

### 2.1 Basic principle

**NucleoSnap® Plasmid Midi** kits are based on a modification of the commonly used and unsurpassed alkaline lysis method that was first described by Birnboim and Doly\*.

*E. coli* cells are grown in a standard culture medium under appropriate selective conditions and harvested by centrifugation.

Cells are resuspended in **Resuspension Buffer SN1** and afterwards lysed by **Lysis Buffer SN2** containing sodium dodecyl sulfate and sodium hydroxide. Alkaline conditions ensure a complete and almost immediate denaturation of DNA and proteins. Addition of **Neutralization Buffer SN3** precipitates potassium dodecyl sulfate complexes with bacterial cell debris, proteins, and macromolecular contaminants and neutralizes the pH value resulting in a re-annealing of the covalently closed circular plasmid DNA which remains soluble.

Debris is removed by a filtration step with the specially designed **NucleoSpin® Plasmid Filter Columns**. The clear flowthrough contains plasmid DNA while genomic DNA, cell remnants, and most of the protein are filtered out and can be discarded.

The flowthrough containing the plasmid DNA is mixed with **Precipitation Buffer SN4** and loaded into a **NucleoSnap® Plasmid Midi Column**, connected to a vacuum device. Vacuum is applied until the solution has passed the filtration matrix completely. Endotoxins are washed away by **Endotoxin Removal Buffer SN5**, salts, and further impurities are subsequently removed by a washing step with ethanolic **Wash Buffer SN6**.

Residual ethanol from Wash Buffer SN6 is efficiently removed by centrifugation in a microcentrifuge. To enable the use of a microcentrifuge, the **NucleoSnap® Columns** are equipped with a predetermined breaking point and can be divided into a funnel component and a Mini spin column by a simple break action.

Plasmid DNA is eluted in **Elution Buffer SNE** (5 mM Tris / HCl, pH 8.5) or other suitable solutions like TE buffer or pure water and is ready for any common downstream application. To achieve an optimal elution efficiency it is required to elute the DNA under neutral to slightly alkaline conditions, ensured by Buffer SNE. No further clean up steps are required.

### 2.2 Kit specifications

The **NucleoSnap® Plasmid Midi** kits are designed for the rapid purification of highly pure plasmid DNA from up to 50 mL of a standard *E. coli* overnight culture. See section 2.5 for possible adaptations to larger culture volumes. Plasmid DNA isolated with this kit is suitable for all common downstream applications like enzymatic digestion, cloning, sequencing, PCR amplification, transformation, and transfection (research use only).

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\* Birnboim, H.C. and Doly, J. (1979) Nucl. Acids Res. 7, 1513–1523

**Table 1: Kit specifications at a glance**

Parameter	NucleoSnap® Plasmid Midi
Sample material	50 mL <i>E. coli</i> culture
Vector size	< 25 kbp
Column capacity	1.5 mg
Typical yield	250 µg (50 mL culture, OD <sub>600</sub> = 4, high-copy plasmid)
Preparation time	30 min/6 samples
Endotoxin level	Transfection-grade
Use	For research use only

## 2.3 Setup of NucleoSnap® Plasmid Columns

**NucleoSnap® Plasmid Columns** are adapted to a vacuum manifold either by a direct Luer connection or by the use of a NucleoVac Mini Adapter or NucleoVac Valves (recommended, see ordering information). 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.

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

The **NucleoSnap® Plasmid Columns** consist of one piece but can be split 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!

It is highly recommended to use a Vacuum regulator (see ordering information) for the adaptation of the required vacuum strength. Excessive vacuum strength will result in reduced plasmid yield. Optimal vacuum strength (-0,3 bar reduction of atmospheric pressure) will filtrate the lysate in approximately 2–3 min.

## 2.4 Reverse pipetting technique

**Precipitation Buffer SN4** 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 Precipitation Buffer SN4 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 Precipitation Buffer SN4 to the cleared lysate, 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.

## 2.5 Estimation of optimal culture volume

The **NucleoSnap® Plasmid Midi** kit is designed for the purification of plasmid DNA from a pellet of *E. coli* cells originating from 50 mL bacterial culture. Nevertheless, the amount of cells per milliliter (titer) varies and depends on many unpredictable factors; therefore, the total amount of pelleted cells varies according to the titer.

Cell lysis depends on the optimal ratio of bacteria to lysing substances. The total amount of the lysing substances sodium dodecyl sulfate and sodium hydroxide is fixed and specified by the volume of Lysis Buffer SN2 added. Lysing substances are consumed during cell lysis, so excess input of bacteria may result in suboptimal lysis and reduced yield.

**As a consequence, the amount of cells is more important for optimal results than the culture volume the cells were pelleted from.**

The titer can easily be estimated by measuring the optical density at 600 nm ( $OD_{600}$ ), blanked against empty culture medium. Due to scattering of light, the  $OD_{600}$  increases according to the number of cells in the optical path with a linear range from about 0.1 to 1. The dilution factor corrected  $OD_{600}$  is directly correlated with the number of cells per volume. Multiplying the  $OD_{600}$  with the pelleted volume gives the ODV number which is relative to the number of cells in a pellet.

Experimental results show a strong correlation between the ODV, added volumes of buffers SN1, SN2, SN3, and plasmid DNA yield. While a high buffer to cell ratio does not have a negative effect, a high cell to buffer ratio decreases yield beyond a maximum cell input. The following formula can be used to calculate the maximal pelleted volume of culture dependent on the bacterial growth ( $OD_{600}$ ) for the lysis conditions of the **NucleoSnap® Plasmid Midi** kit:

$$\frac{250}{OD_{600}} = \text{pelleted culture Volume [mL]}$$

*E.g., if a bacterial culture grew to an  $OD_{600}$  of 5, the pelleted culture volume should not exceed  $250:5 = 50$  mL. With a culture grown to an  $OD_{600}$  of 3, a pelleted volume of  $250:3 \approx 80$  mL would also be possible while a culture grown to an  $OD_{600}$  of 8 would need a decrease in pelleted volume to  $250:8 \approx 30$  mL for optimal results.*

## 2.6 Adaptations for low-copy plasmids

As explained in section 2.5, the amount of cells in relation to the amount of lysis buffer is of crucial importance for optimal results. Increasing the cell input without adapting the lysis buffer volumes accordingly will lead to decreased yield and is not recommended. For working with low-copy plasmids it is usually recommended to use double volumes of bacterial culture and lysis buffers. This is also possible with this kit and will yield similar results.

The **NucleoSpin® Plasmid Filter Columns** are limited in volume though, so if lysis buffer volumes are increased, the **NucleoSpin® Plasmid Filter Columns** cannot be used to clarify the lysates after neutralization. Larger lysate volumes must either be clarified by centrifugation or by filtration.

It is recommended to centrifuge the lysate after neutralization for at least 5 minutes at full speed, to transfer the supernatant into a fresh 50 mL tube (not supplied) and to recentrifuge the samples for at least 5 minutes at full speed. The resulting supernatant must be clear in order to prevent clogging of the NucleoSnap® Plasmid Columns.

Alternatively, a standard gravity flow filtration can be performed with NucleoBond® Folded Filters (see ordering information, section 6.2) that have been equilibrated with 2 mL of Buffer SN3.

Keep in mind that high-copy plasmids are usually present in 100 – 1000 copies per cell while low-copy plasmids are present in 1 – 10 copies per cell only. There is at least a difference of more than factor 10. A difference of factor 2 in cell input will only have a small total effect on yield. To greatly increase yield, all volumes need to be drastically increased.

For processing large culture volumes and thus preparing large amounts of highly concentrated low-copy plasmid DNA the NucleoBond® Xtra or NucleoBond® PC kits are recommended. Contact your local supplier or our technical support at [tech-bio@mn-net.com](mailto:tech-bio@mn-net.com) for more information.

### 3 Storage conditions and preparation of working solutions

- All components can be stored at 15–25 °C and are stable for at least one year.
- Storage of Buffer **SN2** below 20 °C may cause precipitation of sodium dodecyl sulfate. **Check for precipitated salt in Buffer SN2 each time before starting a preparation!** Precipitates might form a firm layer at the bottom of the bottle which is difficult to see from the side or above. Gently invert the bottle a few times (avoid extensive foaming) and carefully inspect the buffer for white flocculates. If salt precipitate is observed, incubate buffer at elevated temperature (e.g., 30–40 °C) for several minutes and mix carefully (avoid extensive foaming) until all precipitate is redissolved completely. Cool down to room temperature before use.
- Dissolve the lyophilized **RNase A** by addition of 3 mL Buffer SN1 to the enzyme vial. Gently swirl the vial or mix by pipetting up and down until the RNase A is dissolved completely. Transfer the RNase A solution back to the bottle containing Buffer SN1 and mix well. Label the addition of RNase A on the check box of Buffer SN1. Store Buffer SN1 with RNase A at 4 °C for up to 6 months. Prepare each Buffer SN1 and RNase A in 740494.50 independently.
- If precipitated salt crystals are visible in Buffer SN5, incubate the Buffer in a tightly closed bottle for 15 minutes at 95 °C and mix well until all precipitates are redissolved.  
Let the buffer cool down to room temperature before use.

Before starting any **NucleoSnap® Plasmid Midi** protocol prepare the following:

- **Wash Buffer SN6:** Add the given volume of ethanol (96–100 %) as indicated on the bottle or in the table below to **Buffer SN6 (Concentrate)** before first use. Mark the label on the bottle to indicate that the ethanol is added. Prepared Buffer SN6 is stable at 15–25 °C for at least one year.

NucleoSnap® Plasmid Midi		
REF	10 preps 740494.10	50 preps 740494.50
Buffer SN6 (Concentrate)	12 mL add 48 mL ethanol	50 mL add 200 mL ethanol

## 4 Safety Instructions

When working with the **NucleoSnap® Plasmid 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 **NucleoSnap® Plasmid 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 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 NucleoSnap® Plasmid Midi protocol

Before starting the preparation:

- Check if RNase A was added to Resuspension Buffer SN1 according to section 3.
- Check Buffer SN2 for precipitates according to section 3.
- Check if Buffer SN6 was prepared according to section 3.
- Recommended: Measure the OD<sub>600</sub> of the culture according to section 2.5.

**All vacuum steps are performed with a reduction of atmospheric pressure of about -0.3 bar\* (10 in. Hg). Do not exceed -0.3 bar (10 in. Hg)!**

### 1 Harvest bacterial cells

Pellet **50 mL *E. coli* culture** by centrifugation at **4,500–6,000 x g** for **≥ 10 min** at **4 °C** and discard the supernatant completely.

**4,500–6,000 x g**  
**4 °C, ≥ 10 min**

*See section 2.5 for recommendations concerning alternative culture volumes dependent on cell titer.*

### 2 Resuspend bacterial cells

Add **5 mL Buffer SN1**. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer SN2!



**+ 5 mL SN1**

### 3 Lyse cells

Add **5 mL Buffer SN2**. Mix gently by inverting the tube 5 times. Do not vortex or pipette!

Incubate at **room temperature (18–25 °C)** for a maximum of **2 min**.



**+ 5 mL SN2**  
**RT, max. 2 min**

### 4 Neutralize

Add **5 mL Buffer SN3**. Mix gently by inverting the tube until the blue color has disappeared completely and an off-white flocculate has formed.



**+ 5 mL SN3**  
**Mix**

\* Reduction of atmospheric pressure.

**5 Clarify lysate**

Transfer the lysate into a **NucleoSpin® Plasmid Filter Column**.

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

*The lysate should pass the column completely. If liquid is remaining on top of the filter membrane, repeat centrifugation until all liquid has passed the filter layers.*

Save the flowthrough. Discard the filter column.



**Load sample**

**3,000 x g,  
2 min**

**Discard  
Plasmid Filter  
Column**

**6 Precipitate DNA**

Add **7 mL Buffer SN4** to the clear flowthrough from step 5.

*The added volume of Buffer SN4 must be about 0.5 volumes of the cleared lysate volume.*

*Note: Reverse pipetting is recommended (see chapter 2.4).*

**Vortex** for **5 s**.



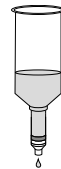
**+ 7 mL SN4**

**Mix**

**7 Filtrate DNA**

Connect the **NucleoSnap® Plasmid Column** to a vacuum manifold and load mixture from step 6 into the column.

Apply **vacuum (-0.3 bar\*)** until the solution has completely passed the filter membrane and then turn vacuum off.



**Load**

**-0.3 bar\***

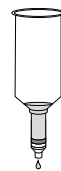
Vacuum

**8 Wash silica membrane**

**1<sup>st</sup> wash**

**Remove endotoxin**

Add **2 mL Buffer SN5** into the NucleoSnap® Plasmid Column. Apply **vacuum (-0.3 bar\*)** until the solution has completely passed the filter membrane and then turn vacuum off.



**+ 2 mL SN5**

**-0.3 bar\***

Vacuum

**2<sup>nd</sup> wash**

**Remove salt**

Add **4 mL Buffer SN6** into the NucleoSnap® Plasmid Column. Apply **vacuum (-0.3 bar\*)** until the solution has completely passed the filter membrane and then turn vacuum off.

**+ 4 mL SN6**

**-0.3 bar\***

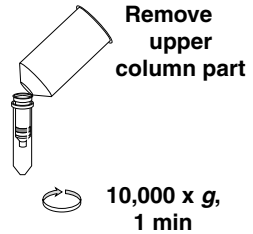
\* Reduction of atmospheric pressure

**9 Dry silica membrane**

Remove the NucleoSnap® Plasmid 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.

Centrifuge Mini spin column and Collection Tube for **1 min** at **> 10,000 x g** to remove any residual ethanol.

Discard the Collection Tube and place the Mini spin column into a new 2 mL Elution Tube (not supplied).



**10 Elute DNA**

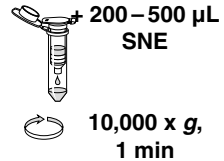
Add **200 µL (high concentration)** or **500 µL (high yield)** of **Elution Buffer SNE** directly to the filter membrane and incubate at **room temperature** for **1 min**.

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

*Optional: Repeat elution with the eluate as elution buffer for optimal recovery.*

*Note: Using 200 µL as elution volume will result in a high DNA concentration but total yield might be reduced.*

*Note: See Chapter 2.1 for possible replacements of Buffer SNE*



## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
No or low DNA yield	<i>No plasmid DNA present in cells</i>
	<ul style="list-style-type: none"> <li>Check plasmid propagation by an alternative plasmid DNA isolation method, e.g., NucleoSpin® Plasmid EasyPure or NucleoSpin® Plasmid.</li> </ul>
	<i>Insufficient resuspension</i>
	<ul style="list-style-type: none"> <li>Completely resuspend the pellet in Buffer SN1. Any remaining cell clumps will be lysed on the surface only, resulting in remaining intact bacteria after lysis.</li> </ul>
	<i>SDS precipitation in Buffer SN2</i>
	<ul style="list-style-type: none"> <li>Check Buffer SN2 for precipitated SDS before adding the buffer to the resuspended cells. Precipitated SDS will result in very low yields.</li> </ul>
	<i>Lysis buffer overloaded</i>
<ul style="list-style-type: none"> <li>If too many cells were harvested, the lysing components will be consumed before sufficient cell lysis. See section 2.5 and check the OD<sub>600</sub> of your culture to prevent an overloading of the lysis system.</li> </ul>	
<i>Insufficient amount of Precipitation Buffer SN4 added.</i>	
<ul style="list-style-type: none"> <li>Buffer SN4 is viscous, make sure to add the correct volume. Use reverse pipetting according to section 2.4 to avoid inaccurate pipetting of precipitation buffer.</li> <li>Precipitation works best when 0.5 vol of Buffer SN4 are added to each vol of cleared lysate.</li> </ul>	
<i>Vacuum force too high</i>	
<ul style="list-style-type: none"> <li>Filtration works best when applied vacuum is at about -0.3 bar* (~ 10 in. Hg). Higher vacuum forces of above -0.7 bar* (~ 20 in. Hg) will result in faster flow rates but also in a loss of DNA.</li> </ul>	
<i>Suboptimal pH of elution solution</i>	
<ul style="list-style-type: none"> <li>Optimal elution requires neutral to slightly alkaline conditions. When using other solutions than the supplied Buffer SNE, check pH and make sure the pH value is at least 7.0</li> </ul>	

\* Reduction of atmospheric pressure.

Problem	Possible cause and suggestions
Slow flow rates	<i>Excess plasmid input</i>
	<ul style="list-style-type: none"> <li>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.</li> </ul>
	<i>Insufficient vacuum force</i>
	<ul style="list-style-type: none"> <li>The lower the vacuum force the slower the flow rate will be. Use vacuum pumps only that enable a minimum of -0.3 bar*.</li> </ul>
Lysate clarification not completely successful	<i>RNA present in cleared lysate</i>
	<ul style="list-style-type: none"> <li>Check if RNase A was added to buffer SN1 according to section 3.</li> </ul>
	<i>Intact cells present in cleared lysate</i>
	<ul style="list-style-type: none"> <li>Remaining cell clumps after resuspension will lead to an incomplete lysis. Intact bacteria will clog the filter.</li> </ul>
	<i>Divergent g-force used during centrifugation</i>
	<ul style="list-style-type: none"> <li>Volumes and times are optimized for 3,000 x g. Lower centrifugal forces result in insufficient lysate clearing, higher centrifugal forces might lead to filter damage.</li> </ul>

## 6.2 Ordering information

Product	REF	Pack
NucleoSnap® Plasmid Midi	740494.10 / .50	10 / 50
NucleoVac 24 Vacuum Manifold	740299	1
NucleoVac Vacuum Regulator	740641	1
Snap Tubes 15 mL	740823.10 / .50	10 / .50
Snap Tubes 50 mL	740822.10 / .50	10 / .50
NucleoVac Mini Adapters	740297.100	100
NucleoVac Valves	740298.24	24
NucleoBond® Folded Filters	740561	5
NucleoSpin® Plasmid	740588.10 / .50 / .250	10 / 50 / 250
NucleoSpin® Plasmid Easy Pure	740727.10 / .50 / .250	10 / 50 / 250

\* Reduction of the atmospheric pressure.

### 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.

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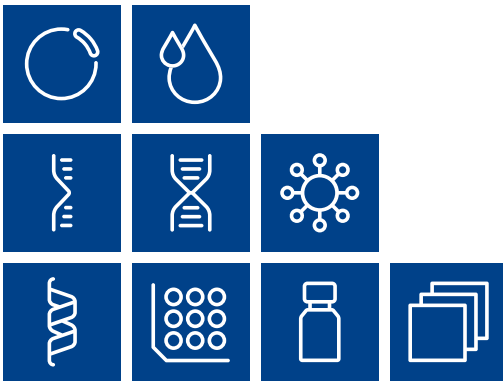
Please contact:  
MACHEREY-NAGEL GmbH & Co. KG  
Tel.: +49 24 21 969-333  
support@mn-net.com

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# MACHEREY-NAGEL

[www.mn-net.com](http://www.mn-net.com)

MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany

DE +49 24 21 969-0 [info@mn-net.com](mailto:info@mn-net.com)

CH +41 62 388 55 00 [sales-ch@mn-net.com](mailto:sales-ch@mn-net.com)

FR +33 388 68 22 68 [sales-fr@mn-net.com](mailto:sales-fr@mn-net.com)

US +1 888 321 62 24 [sales-us@mn-net.com](mailto:sales-us@mn-net.com)

