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Plasmid DNA purification

User manual









NucleoSnap Plasmid Midi

February 2016 / Rev. 01

Plasmid DNA purification

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

NucleoSnap Plasmid Midi

1 Harvest bacterial cells		4,500–6,000 x <i>g</i> 4 °C, ≥ 10 min
2–3 Resuspend bacterial cells and lyse cells		5 mL SN1 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
6 Precipitate DNA		6 mL SN4 Mix
7 Filtrate DNA	 Vacuum	Load -0.3 bar*
8 Wash silica membrane	 Vacuum	1st wash 2 mL SN5 -0.3 bar* 2nd wash 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

NucleoSnap Plasmid Midi		
REF	10 preps 740494.10	50 preps 740494.50
Resuspension Buffer SN1	75 mL	2 x 150 mL
Lysis Buffer SN2	75 mL	2 x 150 mL
Neutralization Buffer SN3	75 mL	2 x 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)*	30 mg	2 x 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
- 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. Please visit the MACHEREY-NAGEL website to verify that you are using the latest revision of this user manual.

* 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 flow-through contains plasmid DNA while genomic DNA, cell remnants, and most of the protein are filtered out and can be discarded.

The flow-through 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) and is ready for any common downstream application. 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).

* 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	400–700 µg (50 mL culture, OD ₆₀₀ = 4, high-copy plasmid)
Preparation time	30 min/6 samples
Endotoxin level	Transfection-grade

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 Stop-cock (recommended, see ordering information). A stop-cock 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!

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 for more information.

3 Storage conditions and preparation of working solutions

- All components can be stored at room temperature (18–25 °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.

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 room temperature (18–25 °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

The following components of the **NucleoSnap Plasmid Midi** kit contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Only harmful features need not to be labeled with H and P phrases until 125 mL or 125 g.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>GHS-Symbol</i>	<i>H-Sätze</i>	<i>P-Sätze</i>
SN2	Sodium hydroxide solution < 2 % <i>Natriumhydroxid-Lösung < 2 %</i> CAS 1310-73-2	 WARNING ACHTUNG	315, 319	264, 280, 302+352, 305+351+338, 332+313, 337+313
SN5	2-propanol 20–50 % <i>2-Propanol 20–50 %</i> CAS 67-63-0	 WARNING ACHTUNG	226, 319, 336	210, 233, 264, 280, 305+351+338, 337+313, 370+378, 403+235
RNase A	RNase A, lyophilized 90–100 % <i>RNase A, lyophilisiert 90–100 %</i> CAS 9001-99-4	 DANGER GEFAHR	317, 334	261, 272, 280, 302+352, 304+340, 333+313, 342+311, 363

Hazard phrases

H 226	Flammable liquid and vapour. <i>Flüssigkeit und Dampf entzündbar.</i>
H 315	Causes skin irritation. <i>Verursacht Hautreizungen.</i>
H 317	May cause an allergic skin reaction. <i>Kann allergische Hautreaktionen verursachen.</i>
H 319	Causes serious eye irritation. <i>Verursacht schwere Augenreizung.</i>
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. <i>Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.</i>
H 336	May cause drowsiness or dizziness. <i>Kann Schläfrigkeit und Benommenheit verursachen.</i>

Precaution phrases

P 210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. <i>Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.</i>
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- P 233 Keep container tightly closed.
Behälter dicht verschlossen halten.
- P 261 Avoid breathing dust/fume/gas/mist/vapours/spray.
Einatmen von Staub/Rauch/Gas/Nebel/Dampf/Aerosol vermeiden.
- P 264 Wash ... thoroughly after handling.
Nach Handhabung ... gründlich waschen.
- P 272 Contaminated work clothing should not be allowed out of the workplace.
Kontaminierte Arbeitskleidung nicht außerhalb des Arbeitsplatzes tragen.
- P 280 Wear protective gloves/protective clothing/eye protection/face protection.
Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz tragen.
- P 302+352 IF ON SKIN: Wash with plenty of water/...
BEI BERÜHRUNG MIT DER HAUT: Mit viel Wasser/... waschen.
- P 304+340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
IF INHALED: Remove person to fresh air and keep comfortable for breathing.
- P 305+351+338 IF IN EYES: Rinse cautiously with water for several mins. Remove contact lenses, if present and easy to do. Continue rinsing.
BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser ausspülen. Eventuell vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.
- P 332+313 If skin irritation occurs: Get medical advice/attention.
If skin irritation occurs: Get medical advice/attention.
- P 333+313 If skin irritation or rash occurs: Get medical advice/attention.
If skin irritation or rash occurs: Get medical advice/attention.
- P 337+313 If eye irritation persists: Get medical advice/attention.
If eye irritation persists: Get medical advice/attention.
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor/...
If experiencing respiratory symptoms: Call a POISON CENTER/doctor/...
- P 363 Wash contaminated clothing before reuse.
Kontaminierte Kleidung vor erneutem Tragen waschen.
- P 370+378 In case of fire: Use ... to extinguish.
In case of fire: Use ... to extinguish.
- P 403+235 Store in a well-ventilated place. Keep cool.
An einem gut belüfteten Ort aufbewahren. Kühl halten.

For further information please see Material Safety Data Sheets (www.mn-net.com).
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (www.mn-net.com).



The symbol shown on labels refers to further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

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 OD₆₀₀ 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).

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.

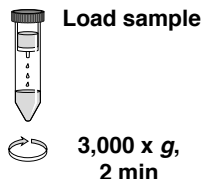
5 Clarify lysate

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 flow-through. Discard the filter column.



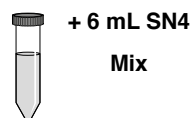
6 Precipitate DNA

Add **6 mL Buffer SN4** to the clear flow-through 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).

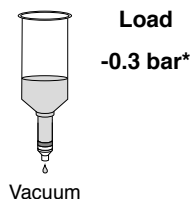
Vortex for **5 s**.



7 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.



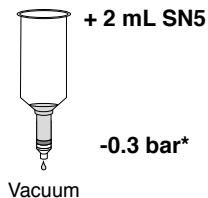
* Reduction of atmospheric pressure

8 Wash silica membrane

1st 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.



2nd wash

Remove salt

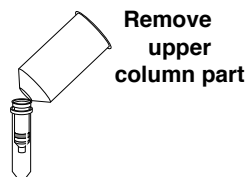
Add **4 mL Buffer SN6** into the NucleoSnap Plasmid Column. Apply **vacuum (-0.3 bar*)** until the solution has completely passed the filter membrane and then turn vacuum off.

+ 4 mL SN6

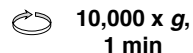
-0.3 bar*

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.



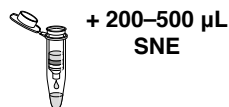
Centrifuge Mini spin column and Collection Tube for **1 min** at **> 10,000 x g** to remove any residual ethanol.



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.

* Reduction of atmospheric pressure

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or low DNA yield	<p data-bbox="333 309 661 333"><i>No plasmid DNA present in cells</i></p> <ul data-bbox="333 352 960 432" style="list-style-type: none"> <li data-bbox="333 352 960 432">• Check plasmid propagation by an alternative plasmid DNA isolation method, e.g., NucleoSpin® Plasmid EasyPure or NucleoSpin® Plasmid.
	<p data-bbox="333 448 586 472"><i>Insufficient resuspension</i></p> <ul data-bbox="333 491 922 571" style="list-style-type: none"> <li data-bbox="333 491 922 571">• 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.
	<p data-bbox="333 587 654 611"><i>SDS precipitation in Buffer SN2</i></p> <ul data-bbox="333 630 969 710" style="list-style-type: none"> <li data-bbox="333 630 969 710">• Check Buffer SN2 for precipitated SDS before adding the buffer to the resuspended cells. Precipitated SDS will result in very low yields.
No or low DNA yield	<p data-bbox="333 726 572 750"><i>Lysis buffer overloaded</i></p> <ul data-bbox="333 769 978 879" style="list-style-type: none"> <li data-bbox="333 769 978 879">• If too many cells were harvested, the lysing components will be consumed before sufficient cell lysis. See section 2.5 and check the OD₆₀₀ of your culture to prevent an overloading of the lysis system.
	<p data-bbox="333 895 874 919"><i>Insufficient amount of Precipitation Buffer SN4 added.</i></p> <ul data-bbox="333 938 975 1094" style="list-style-type: none"> <li data-bbox="333 938 975 1023">• 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 data-bbox="333 1038 922 1094">• Precipitation works best when 0.5 vol of Buffer SN4 are added to each vol of cleared lysate.
	<p data-bbox="333 1107 561 1131"><i>Vacuum force too high</i></p> <ul data-bbox="333 1150 969 1267" style="list-style-type: none"> <li data-bbox="333 1150 969 1267">• 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.

* Reduction of atmospheric pressure.

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

6.2 Ordering information

Product	REF	Pack
NucleoSnap Plasmid Midi	740494.10/.50	10/ 50
NucleoVac 24 Vacuum Manifold	740299	1
NucleoVac Mini Adapter	740297.100	100
NucleoVac Stop-cock	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

NucleoSnap Plasmid 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

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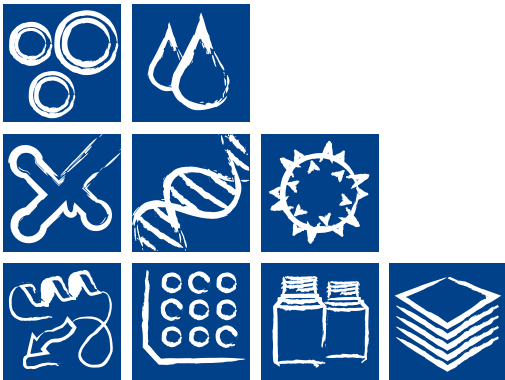
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