

Plasmid DNA purification

User manual

NucleoSpin[®] Plasmid

NucleoSpin[®] Plasmid (NoLid)

NucleoSpin[®] Plasmid QuickPure

March 2019 / Rev. 11

MACHERY-NAGEL

www.mn-net.com



DOMINIQUE DUTSCHER SAS

Plasmid DNA purification

Protocol at a glance (Rev.11)

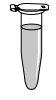
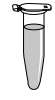
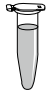




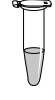


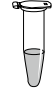




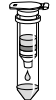
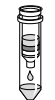
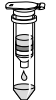



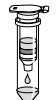
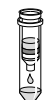














	NucleoSpin® Plasmid	NucleoSpin® Plasmid (NoLid)		NucleoSpin® Plasmid QuickPure
1 Cultivate and harvest bacterial cells				
			11,000 x g, 30 s	
2 Cell lysis			250 µL Buffer A1 250 µL Buffer A2 RT, up to 5 min 300 µL Buffer A3	
3 Clarification of the lysate				
			11,000 x g, 5–10 min	
4 Bind DNA			Load supernatant	
			11,000 x g, 1 min	
5 Wash silica membrane			<i>(Optional: 500 µL Buffer AW: RT or 50 °C)</i> 600 µL Buffer A4	
			11,000 x g, 1 min	
6 Dry silica membrane				
			11,000 x g, 2 min	Drying is performed during centrifugation of the single washing step
7 Elute DNA			50 µL Buffer AE RT, 1 min	
			11,000 x g, 1 min	



Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	7
1.3	About this user manual	7
2	Product description	8
2.1	Basic principle	8
2.2	Kit specifications	8
2.3	Growth of bacterial cultures	9
2.4	Lysate neutralization and LyseControl	11
2.5	Elution procedures	11
3	Storage conditions and preparation of working solutions	12
4	Safety instructions	13
5	NucleoSpin® Plasmid/Plasmid (NoLid) protocols	15
5.1	Isolation of high-copy plasmid DNA from <i>E. coli</i>	15
5.2	Isolation of low-copy plasmids, P1 constructs, or cosmids	17
6	NucleoSpin® Plasmid QuickPure protocol – isolation of high-copy plasmid DNA from <i>E. coli</i>	19
7	NucleoSpin® Plasmid/Plasmid (NoLid), and NucleoSpin® Plasmid QuickPure protocols	21
7.2	Plasmid DNA clean up	22
8	Appendix	23
8.1	Troubleshooting	23
8.2	Ordering information	26
8.3	References	26
8.4	Product use restriction/warranty	26

1 Components

1.1 Kit contents

REF	NucleoSpin® Plasmid		
	10 preps 740588.10	50 preps 740588.50	250 preps 740588.250
Resuspension Buffer A1	5 mL	15 mL	75 mL
Lysis Buffer A2	5 mL	15 mL	100 mL
Neutralization Buffer A3	5 mL	20 mL	100 mL
Wash Buffer AW	6 mL	30 mL	2 x 75 mL
Wash Buffer A4 (Concentrate)*	6 mL	12 mL	2 x 25 mL
Elution Buffer AE**	13 mL	13 mL	60 mL
RNase A (lyophilized)*	2.5 mg	6 mg	30 mg
NucleoSpin® Plasmid Columns (white rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
User manual	1	1	1

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

** Composition of Elution Buffer AE: 5 mM Tris/HCl, pH 8.5

Kit contents *continued*

REF	NucleoSpin® Plasmid (NoLid)		
	10 preps 740499.10	50 preps 740499.50	250 preps 740499.250
Resuspension Buffer A1	5 mL	15 mL	75 mL
Lysis Buffer A2	5 mL	15 mL	100 mL
Neutralization Buffer A3	5 mL	20 mL	100 mL
Wash Buffer AW	6 mL	30 mL	2 x 75 mL
Wash Buffer A4 (Concentrate)*	6 mL	12 mL	2 x 25 mL
Elution Buffer AE**	13 mL	13 mL	60 mL
RNase A (lyophilized)*	2.5 mg	6 mg	30 mg
NucleoSpin® Plasmid (NoLid) Columns (white rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
User manual	1	1	1

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

** Composition of Elution Buffer AE: 5 mM Tris/HCl, pH 8.5

Kit contents *continued*

NucleoSpin® Plasmid QuickPure			
REF	10 preps 740615.10	50 preps 740615.50	250 preps 740615.250
Resuspension Buffer A1	5 mL	15 mL	75 mL
Lysis Buffer A2 (without LyseControl)	5 mL	15 mL	100 mL
Neutralization Buffer A3	5 mL	20 mL	100 mL
Wash Buffer AQ (Concentrate)*	6 mL	6 mL	25 mL
Elution Buffer AE**	13 mL	13 mL	60 mL
RNase A (lyophilized)*	2.5 mg	6 mg	30 mg
NucleoSpin® Plasmid QuickPure Columns (dark yellow rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
User manual	1	1	1

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

** Composition of Elution Buffer AE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol

Consumables

- 1.5 mL microcentrifuge tubes for sample lysis and DNA elution
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Heating-block (NucleoSpin® Plasmid/Plasmid (NoLid): for large constructs or optional Wash Buffer AW)
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the user manual **NucleoSpin® Plasmid/Plasmid (NoLid)** or **NucleoSpin® Plasmid QuickPure** before using these products. 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 on the Internet at www.mn-net.com. Please visit the MACHEREY-NAGEL website to verify that you are using the latest revision of this user manual.

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

2 Product description

2.1 Basic principle

With the **NucleoSpin® Plasmid** method, the pelleted bacteria are resuspended (Buffer A1) and plasmid DNA is liberated from the *E. coli* host cells by SDS / alkaline lysis (Buffer A2). Buffer A3 neutralizes the resulting lysate and creates appropriate conditions for binding of plasmid DNA to the silica membrane of the **NucleoSpin® Plasmid/Plasmid (NoLid)** or **NucleoSpin® Plasmid QuickPure Column**. Precipitated protein, genomic DNA, and cell debris are then pelleted by a centrifugation step. The supernatant is loaded onto a **NucleoSpin® Plasmid/Plasmid (NoLid)** or **NucleoSpin® Plasmid QuickPure Column**.

With the **NucleoSpin® Plasmid/Plasmid (NoLid)** kit contaminations like salts, metabolites, and soluble macromolecular cellular components are removed by simple washing with ethanolic Buffer A4. Pure plasmid DNA is finally eluted under low ionic strength conditions with slightly alkaline Buffer AE (5 mM Tris/HCl, pH 8.5). If host strains with high levels of nucleases are used, an additional washing step with preheated Buffer AW is recommended. Additional washing with Buffer AW will also increase the reading length of automated fluorescent DNA sequencing reactions.

With the **NucleoSpin® Plasmid QuickPure** kit contaminations like salts, metabolites, nucleases, and soluble macromolecular cellular components are removed by only a single washing step with Buffer AQ. Pure plasmid DNA is finally eluted under low ionic strength conditions with slightly alkaline Buffer AE (5 mM Tris/HCl, pH 8.5).

2.2 Kit specifications

- The **NucleoSpin® Plasmid/Plasmid (NoLid)** and **NucleoSpin® Plasmid QuickPure** kits are designed for the rapid, small-scale preparation of highly pure plasmid DNA (mini preps).
- The **NucleoSpin® Plasmid/Plasmid (NoLid) Columns** offer a very high DNA binding capacity of up to 60 µg. This, however, requires thorough washing. Therefore, the kit includes an additional Wash Buffer AW which is strongly recommended for host strains with high levels of endonucleases like ABLE, HB101, or JM110.
- The **NucleoSpin® Plasmid QuickPure Column** features a new specially treated silica membrane which allows speeding up the procedure by a combined washing and drying step. No additional steps are necessary if nuclease rich host strains are used. The number of washing and drying steps is reduced from 3 to only 1! Therefore, the hands-on time is less than 11 min. However, the DNA binding capacity is limited to 15 µg.
- The plasmid DNA prepared with both kits, **NucleoSpin® Plasmid/Plasmid (NoLid)** and **NucleoSpin® Plasmid QuickPure**, is suitable for applications like automated fluorescent DNA sequencing, PCR, or any kind of enzymatic manipulation.

- Furthermore, support protocols allow purification of low-copy plasmids from larger culture volumes, purification of plasmids from Gram positive bacteria, and clean up of plasmids from reaction mixtures.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Plasmid / Plasmid (NoLid)	NucleoSpin® Plasmid QuickPure
Culture volume	1–5 mL (high copy) 6–10 mL (low copy)	1–3 mL (high copy)
Typical yield	<25 µg (1–5 mL culture) <45 µg (6–10 mL culture)	< 15 µg (1–3 mL culture)
Elution volume	50 µL	50 µL
Binding capacity	60 µg	15 µg
Vectors	<25 kbp	< 15 kbp
Preparation time*	20 min/6 preps	11 min/6 preps
Format	Mini spin column	Mini spin column

2.3 Growth of bacterial cultures

Yield and quality of plasmid DNA highly depend on the type of culture media and antibiotics, the bacterial host strain, the plasmid type, size, or copy number.

For cultivation of bacterial cells harbouring standard high-copy plasmids, we recommend **LB (Luria Bertani) medium**. The cell culture should be incubated at 37 °C with constant shaking (200–250 rpm) preferably 12–16 h over night. Usually an OD of 3–6 can be achieved. Alternatively, rich media like 2x YT (Yeast/Tryptone), TB (Terrific Broth), or CircleGrow can be used. In this case bacteria grow faster, reach the stationary phase much earlier than in LB medium (≤ 12 h), and higher cell masses can be reached. However, this does not necessarily yield more plasmid DNA. Overgrowing a culture might lead to a higher percentage of dead or starving cells and the resulting plasmid DNA might be partially degraded or contaminated with chromosomal DNA. To find the optimal culture conditions, the culture medium and incubation times have to be optimized for each host strain/plasmid construct combination individually.

Cell cultures should be grown under antibiotic selection at all times to ensure plasmid propagation. Without this selective pressure, cells tend to lose a plasmid during cell division. Since bacteria grow much faster without the burden of a high-copy plasmid, they take over the culture rapidly and the plasmid yield decreases regardless of the cell mass. Table 2 gives information on concentrations of commonly used antibiotics.

* Hands-on-time

Table 2: Information about antibiotics according to Maniatis*

Antibiotic	Stock solution (concentration)	Storage	Working concentration
Ampicillin	50 mg/mL in H ₂ O	-20 °C	20–50 µg/mL
Carbenicillin	50 mg/mL in H ₂ O	-20 °C	20–60 µg/mL
Chloramphenicol	34 mg/mL in EtOH	-20 °C	25–170 µg/mL
Kanamycin	10 mg/mL in H ₂ O	-20 °C	10–50 µg/mL
Streptomycin	10 mg/mL in H ₂ O	-20 °C	10–50 µg/mL
Tetracycline	5 mg/mL in EtOH	-20 °C	10–50 µg/mL

As rule of thumb use 5 mL of a well grown culture for **NucleoSpin® Plasmid/Plasmid (NoLid)** and 3 mL for **NucleoSpin® Plasmid QuickPure** as given in the kit specifications.

However, the culture volume can be increased if the cell culture grows very poorly or has to be decreased if e.g. very rich culture media were used. Refer to Table 3 and Table 4 to choose the best culture volume according to the optical density at 600 nm (OD₆₀₀).

Table 3: Recommended culture volumes for NucleoSpin® Plasmid/Plasmid (NoLid)

OD ₆₀₀	1	2	3	4	5	6
Culture volume (high copy)	15 mL	8 mL	5 mL	4 mL	3 mL	2 mL
Culture volume (low copy)**	–	–	10 mL	8 mL	6 mL	4 mL

Table 4: Recommended culture volumes for NucleoSpin® Plasmid QuickPure

OD ₆₀₀	1	2	3	4	5	6
Culture volume	8 mL	4 mL	3 mL	2 mL	1 mL	1 mL

Note, if too much bacterial material is used, the lysis and precipitation steps become inefficient causing decreased yield and plasmid quality! If more than the recommended amount of cells shall be processed refer to the support protocol for low-copy plasmid purification (section 5.2).

* Maniatis T, Fritsch EF, Sambrook J: *Molecular cloning. A laboratory manual*, Cold Spring Harbor, Cold Spring, New York 1982.

** Please follow the procedure for low-copy plasmids, see section 5.2.

2.4 Lysate neutralization and LyseControl

Proper mixing of the lysate with Neutralization Buffer A3 is of utmost importance for complete precipitation of SDS, protein, and genomic DNA. Incomplete neutralization leads to reduced yield. However, released plasmid DNA is very vulnerable at this point and shaking too much or too strongly will damage the DNA.

Therefore, do not vortex or shake but just invert the mixture very gently until a fluffy off-white precipitate has formed and the blue LyseControl in Buffer A2 has turned colorless throughout the lysate without any traces of blue color.

2.5 Elution procedures

The elution buffer volume and method can be adapted to the subsequent downstream application to achieve higher yield and/or concentration than the standard method (recovery about 70–90 %):

- **Higher yield in general, especially for larger constructs:** Heat elution buffer to 70 °C, add 50–100 µL to the NucleoSpin® Plasmid/Plasmid (NoLid) Column and incubate at 70 °C for 2 min.
- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90–100 % of bound nucleic acids can be eluted.
- **High concentration:** Perform one elution step with 60 % of the volume indicated in the individual protocol. Concentration of DNA will be higher than with standard elution (approx. 130 %). Maximal yield of bound nucleic acids is about 80 %.
- **High yield and high concentration:** Apply half of the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate, and centrifuge again. Thus, about 85–100 % of bound nucleic acids are eluted with the standard elution volume at a high concentration.

Elution Buffer AE (5 mM Tris/HCl, pH 8.5) can be replaced by TE buffer or water as well. However, we recommend using a weakly buffered, slightly alkaline buffer containing no EDTA, especially if the plasmid DNA is intended for sequencing reactions. If water is used, the pH should be checked and adjusted to pH 8.0–8.5 since deionized water usually exhibits a pH below 7. Furthermore absorption of CO₂ leads to a decrease in pH of unbuffered solutions.

3 Storage conditions and preparation of working solutions

Attention: Buffer A3 and Buffer AW contain guanidine hydrochloride! Wear gloves and goggles!

CAUTION: Buffers A3 and AW contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample preparation waste.

- All kit components can be stored at room temperature (18–25 °C) and are stable for at least one year.
- Always keep buffer bottles tightly closed, especially if buffers are preheated during the preparation.
- Sodium dodecyl sulfate (SDS) in Buffer A2 may precipitate if stored at temperatures below 20 °C. If a precipitate is observed in Buffer A2, incubate the bottle at 30–40 °C for several minutes and mix well.

Before starting any **NucleoSpin® Plasmid/Plasmid (NoLid)** or **NucleoSpin® Plasmid QuickPure** protocol prepare the following:

- Add 1 mL of Buffer A1 to the RNase A vial and vortex. Transfer the solution back into the Buffer A1 bottle and mix thoroughly. Indicate date of RNase A addition. Store Buffer A1 containing RNase A at 4 °C. The solution will be stable at this temperature for at least six months.
- Add the indicated volume of 96–100 % ethanol to Buffer A4 and Buffer AQ.

NucleoSpin® Plasmid/Plasmid (NoLid)

	10 preps 740588.10/ 740499.10	50 preps 740588.50/ 740499.50	250 preps 740588.250/ 740499.250
REF			
Wash Buffer A4 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	2 x 25 mL Add 100 mL ethanol to each bottle

NucleoSpin® Plasmid Quick Pure

	10 preps 740615.10	50 preps 740615.50	250 preps 740615.250
REF			
Wash Buffer AQ (Concentrate)	6 mL Add 24 mL ethanol	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol





4 Safety instructions

The following components of the **NucleoSpin® Plasmid/Plasmid (NoLid)** and **NucleoSpin® Plasmid QuickPure** kits contain hazardous contents.

GHS classification

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

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Inhalt	Gefahrstoff	GHS-Symbol	H-Sätze	P-Sätze
A2	Sodium hydroxide solution 0,5–1,0 % <i>Natriumhydroxid-Lösung</i> 0,5–1,0 % CAS 1310-73-2	 WARNING ACHTUNG	315, 319	280sh
A3	Guanidine hydrochloride 36–50 % <i>Guanidinhydrochlorid 36–50 %</i> CAS 50-01-1	 WARNING ACHTUNG	302, 319	264W, 280sh, 301+312, 330
AW	Guanidine hydrochloride 36–50 % and 2-propanol 20–35 % <i>Guanidinhydrochlorid 36–50 %</i> <i>und 2-Propanol 20–35 %</i> CAS 50-01-1, 67-63-0	 WARNING ACHTUNG	226, 302, 319, 336	210, 260D, 264W, 280sh, 301+312, 330
RNase A	RNase 90–100 %	 DANGER GEFAHR	334	261sh, 342+311

Hazard phrases

H 226	Flammable liquid and vapour. <i>Flüssigkeit und Dampf entzündbar.</i>
H 302	Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i>
H 315	Causes skin irritation. <i>Verursacht Hautreizungen.</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.
Kann Schläfrigkeit und Benommenheit verursachen.

Precaution phrases

P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.

P 260D Do not breathe vapors.
Dampf nicht einatmen.

P 261sh Avoid breathing dust / vapors.
Einatmen von Staub / Dampf vermeiden.

P 264W Wash with water thoroughly after handling.
Nach Gebrauch mit Wasser gründlich waschen.

P 280sh Wear protective gloves/eye protection.
Schutzhandschuhe / Augenschutz tragen.

P 301+312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt anrufen.

P 330 Rinse mouth.
Mund ausspülen.

P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER / doctor.
Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM/Arzt anrufen.



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.

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

5 NucleoSpin® Plasmid / Plasmid (NoLid) protocols

5.1 Isolation of high-copy plasmid DNA from *E. coli*

Before starting the preparation:

- Check if Wash Buffer A4 was prepared according to section 3.

1 Cultivate and harvest bacterial cells

Use **1–5 mL** of a saturated ***E. coli* LB culture**, pellet cells in a standard benchtop microcentrifuge for **30 s** at **11,000 x g**. Discard the supernatant and remove as much of the liquid as possible.



**11,000 x g,
30 s**

Note: For isolation of low-copy plasmids refer to section 5.2.

2 Cell lysis

Add **250 µL Buffer A1**. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer A2!

**+ 250 µL A1
Resuspend**

Attention: Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30–40 °C until precipitate is dissolved completely. Mix thoroughly and cool buffer down to room temperature (18–25 °C).



**+ 250 µL A2
Mix
RT, 5 min**

Add **250 µL Buffer A2**. Mix gently by inverting the tube **6–8 times**. Do not vortex to avoid shearing of genomic DNA. Incubate at **room temperature** for up to **5 min** or until lysate appears clear.

Add **300 µL Buffer A3**. Mix thoroughly by inverting the tube **6–8 times** until blue samples turn colorless completely! Do not vortex to avoid shearing of genomic DNA!

**+ 300 µL A3
Mix**

Make sure to neutralize completely to precipitate all protein and chromosomal DNA. LyseControl should turn completely colorless without any traces of blue.

3 Clarification of lysate

Centrifuge for **5 min** at **11,000 x g** at room temperature. Repeat this step in case the supernatant is not clear!



**11,000 x g,
5–10 min**

4 Bind DNA

Place a NucleoSpin® Plasmid/Plasmid (NoLid) Column in a Collection Tube (2 mL) and decant the supernatant from step 3 or pipette a maximum of 700 µL of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flowthrough and place the NucleoSpin® Plasmid/Plasmid (NoLid) Column back into the collection tube.



**Load
supernatant**



**11,000 x g,
1 min**

Repeat this step to load the remaining lysate.

5 Wash silica membrane

Recommended: If plasmid DNA is prepared from host strains containing high levels of nucleases (e.g., HB101 or strains of the JM series), **it is strongly recommended** performing an additional washing step with **500 µL Buffer AW, optionally preheated to 50 °C**, and centrifuge for **1 min** at **11,000 x g** before proceeding with Buffer A4. Additional washing with Buffer AW will also increase the reading length of DNA sequencing reactions and improve the performance of critical enzymatic reactions.



**Optional:
+ 500 µL AW**



**11,000 x g,
1 min**

Add **600 µL Buffer A4** (supplemented with ethanol, see section 3). Centrifuge for **1 min** at **11,000 x g**. Discard flowthrough and place the NucleoSpin® Plasmid/Plasmid (NoLid) Column back into the **empty** collection tube.



+ 600 µL A4

**11,000 x g,
1 min**

6 Dry silica membrane

Centrifuge for **2 min** at **11,000 x g** and discard the collection tube.



Note: Residual ethanolic wash buffer might inhibit enzymatic reactions.



**11,000 x g,
2 min**

7 Elute DNA

Place the NucleoSpin® Plasmid/Plasmid (NoLid) Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL Buffer AE**. Incubate for **1 min** at **room temperature**. Centrifuge for **1 min** at **11,000 x g**.



**+ 50 µL AE
RT, 1 min**



**11,000 x g,
1 min**

Note: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see section 2.5.

5.2 Isolation of low-copy plasmids, P1 constructs, or cosmids

Processing of larger culture volumes requires increased lysis buffer volumes. The buffer volumes provided with the kit are calculated for high-copy plasmid purification only. Thus, if this support protocol is to be used frequently, an additional NucleoSpin® Buffer Set can be ordered separately (see ordering information, section 8.2).

Before starting the preparation:

- Check if Wash Buffer A4 was prepared according to section 3.

1 Cultivate and harvest bacterial cells

Use **5–10 mL** of a saturated *E.coli* **LB culture**, pellet cells in a standard benchtop microcentrifuge for 30 s at **11,000 x g**. Discard the supernatant and remove as much of the liquid as possible.



**11,000 x g,
30 s**

2 Cell lysis

Add **500 µL Buffer A1**. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer A2!

+ 500 µL A1

Resuspend

Attention: Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30–40 °C until precipitate is dissolved completely. Mix thoroughly and cool buffer down to room temperature (18–25 °C).



+ 500 µL A2

Mix

RT, 5 min

Add **500 µL Buffer A2**. Mix gently by inverting the tube **6–8 times**. Do not vortex to avoid shearing of genomic DNA. Incubate at **room temperature** for up to **5 min** or until lysate appears clear.

+ 600 µL A3

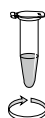
Mix

Add **600 µL Buffer A3**. Mix thoroughly by inverting the tube **6–8 times** until blue samples turn colorless completely! Do not vortex to avoid shearing of genomic DNA!

Make sure to neutralize completely to precipitate all the protein and chromosomal DNA. LyseControl should turn completely colorless without any traces of blue.

3 Clarification of lysate

Centrifuge for **10 min** at **11,000 x g** at room temperature



**11,000 x g,
5–10 min**

4 Bind DNA

Place a NucleoSpin® Plasmid/Plasmid (NoLid) Column in a Collection Tube (2 mL) and decant the supernatant from step 3 or pipette a maximum of 700 µL of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flowthrough and place the NucleoSpin® Plasmid Column back into the collection tube.



Load supernatant

**11,000 x g,
1 min**

Repeat this step to load the remaining lysate.

5 Wash silica membrane

Recommended: Add **500 µL Buffer AW**, optionally **preheated to 50 °C**, and centrifuge for **1 min** at **11,000 x g**. Discard flowthrough and place the NucleoSpin® Plasmid/Plasmid (NoLid) Column back into the collection tube.



**Optional:
+ 500 µL AW**

**11,000 x g,
1 min**

Add **600 µL Buffer A4** (supplemented with ethanol, see section 3). Centrifuge for **1 min** at **11,000 x g**. Discard flowthrough and place the NucleoSpin® Plasmid/Plasmid (NoLid) Column back into the empty collection tube.



+ 600 µL A4

**11,000 x g,
1 min**

6 Dry silica membrane

Centrifuge for **2 min** at **11,000 x g** and discard the collection tube.



Note: Residual ethanolic wash buffer might inhibit enzymatic reactions.



**11,000 x g,
2 min**

7 Elute DNA

Place the NucleoSpin® Plasmid/Plasmid (NoLid) Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL Buffer AE preheated to 70 °C**. Incubate for 2 min at **70 °C**. Centrifuge for **1 min** at **11,000 x g**.



**+ 50 µL AE
RT, 1 min**

Note: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see section 2.5



**11,000 x g,
1 min**

6 NucleoSpin® Plasmid QuickPure protocol – isolation of high-copy plasmid DNA from *E. coli*

Before starting the preparation:

- Check if Wash Buffer AQ was prepared according to section 3.

1 Cultivate and harvest bacterial cells

Use **1–3 mL** of a saturated *E. coli* **LB culture**, pellet cells in a standard benchtop microcentrifuge for 30 s at **11,000 x g**. Discard the supernatant and remove as much of the liquid as possible.



**11,000 x g,
30 s**

2 Cell lysis

Add **250 µL Buffer A1**. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer A2!

**+ 250 µL A1
Resuspend**

Attention: Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30–40 °C until precipitate is dissolved completely. Mix thoroughly and cool buffer down to room temperature (18–25 °C).



**+ 250 µL A2
Mix
RT, 5 min**

Add **250 µL Buffer A2**. Mix gently by inverting the tube **6–8 times**. Do not vortex to avoid shearing of genomic DNA. Incubate at **room temperature** for up to **5 min** or until lysate appears clear.

**+ 300 µL A3
Mix**

Add **300 µL Buffer A3**. Mix thoroughly by inverting the tube **6–8 times** until blue samples turn colorless completely! Do not vortex to avoid shearing of genomic DNA!

Make sure to neutralize completely to precipitate all the protein and chromosomal DNA.

3 Clarification of lysate

Centrifuge for **5 min** at **11,000 x g** at room temperature.



**11,000 x g,
5–10 min**

4 Bind DNA

Place a NucleoSpin® Plasmid QuickPure Column in a Collection Tube (2 mL) and decant the supernatant from step 3 or pipette a maximum of 700 µL of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flowthrough and place the NucleoSpin® Plasmid QuickPure Column back into the collection tube.

**Load supernatant****11,000 x g,
1 min**

Repeat this step to load the remaining lysate.

5 Wash silica membrane

Add **450 µL Buffer AQ** (supplemented with ethanol, see section 3). Centrifuge for **3 min** at **11,000 x g**.

**+ 450 µL AQ**

Very carefully discard the collection tube and the flowthrough and make sure the spin cup outlet does not touch the wash buffer surface. Otherwise repeat the centrifugation step.

**11,000 x g,
3 min****6 Dry silica membrane**

The drying of the NucleoSpin® Plasmid QuickPure Column is performed by the 3 min centrifugation in step 5.

7 Elute DNA

Place the NucleoSpin® Plasmid QuickPure Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL Buffer AE**. Incubate for 1 min at **room temperature**. Centrifuge for **1 min** at **11,000 x g**.

**+ 50 µL AE****RT, 1 min**

Note: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see section 2.5.

**11,000 x g,
1 min**

7 NucleoSpin® Plasmid/Plasmid (NoLid), and NucleoSpin® Plasmid QuickPure protocols

7.1 Isolation of plasmids from Gram positive bacteria

For plasmid purification from bacteria with a more resistant cell wall (e.g., *Bacillus*, *Staphylococcus*), it is necessary to start the lysis procedure with an enzymatic treatment (e.g., Lysozyme, Lysostaphin, Mutanolysin) to break up the peptidoglycan layers.

For some Gram positive bacteria (e.g., *Bifidobacteria*, *Corynebacteria*) even a preincubation with lysozyme might be insufficient and mechanical cell disruption methods have to be used (e.g., RiboLyser).

Before starting the preparation:

- Check if Wash Buffer A4 or Buffer AQ were prepared according to section 3.

1 Cultivate and harvest bacterial cells

Use up to **5 mL** (NucleoSpin® Plasmid/Plasmid (NoLid)) or **3 mL** (NucleoSpin® Plasmid QuickPure) of a saturated ***E. coli* LB culture**, pellet cells in a standard benchtop microcentrifuge for **30 s** at **11,000 x g**. Discard the supernatant and remove as much liquid as possible.



**11,000 x g,
30 s**

2 Cell lysis

Add **250 µL Buffer A1** containing **10 mg/mL Lysozyme** (not provided with the kit). Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain in the suspension!

**+ 250 µL A1
+ Lysozyme**

Resuspend

Incubate at **37 °C** for **10–30 minutes**.

Proceed with addition of Buffer A2 in step 2 of the protocol for isolation of high-copy plasmids from *E. coli* with NucleoSpin® Plasmid/Plasmid (NoLid) (section 5.1) or NucleoSpin® Plasmid QuickPure (section 6).

**37 °C,
10–30 min**

7.2 Plasmid DNA clean up

Plasmid or DNA fragment preparations from other origins than bacterial cells, for example, enzymatic reactions, can be purified using NucleoSpin® Plasmid/Plasmid (NoLid) or Plasmid QuickPure by omitting the cell lysis step.

Before starting the preparation:

- Check if Wash Buffer A4 or Buffer AQ were prepared according to section 3.

1 Adjust binding condition

Add 2 volumes of Buffer A3 to 1 volume of DNA solution and mix well by vortexing.



+ 2 vol A3
Mix

(For example, add 200 µL Buffer A3 to 100 µL enzymatic reaction mix.)

2 Bind DNA

Place a NucleoSpin® Plasmid/Plasmid (NoLid) or NucleoSpin® Plasmid QuickPure Column in a Collection Tube (2 mL) and load the mixture onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flowthrough and place the NucleoSpin® Plasmid/Plasmid (NoLid) Column or Plasmid QuickPure Column back into the collection tube.



Load mixture



11,000 x g,
1 min

Note: Maximum loading capacity of the NucleoSpin® Plasmid/Plasmid (NoLid) Column or Plasmid QuickPure Column is 700 µL. Repeat the procedure if larger volumes are to be processed.

Proceed with the washing step 5 of the protocol for isolation of high-copy plasmids from *E.coli* with NucleoSpin® Plasmid/Plasmid (NoLid) (section 5.1) or NucleoSpin® Plasmid QuickPure (section 6).

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of bacterial cells	<p><i>Cell pellet not properly resuspended</i></p> <p>It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of Buffer A2.</p> <p><i>SDS in Buffer A2 precipitated</i></p> <p>SDS in Buffer A2 may precipitate upon storage. If a precipitate is formed, incubate Buffer A2 at 30–40 °C for 5 min and mix well.</p>
	<p><i>Too many bacterial cells used</i></p> <p>We recommend LB as optimal growth medium. When using very rich media like TB (terrific broth), the cell density of the cultures may become too high.</p>
Poor plasmid yield	<p><i>Incomplete lysis of bacterial cells</i></p> <ul style="list-style-type: none"> • See „Possible cause and suggestions“ above. <p><i>Suboptimal precipitation of SDS and cell debris</i></p> <ul style="list-style-type: none"> • Precipitation of SDS and cell debris will be slightly more effective when centrifuging at 4 °C instead of room temperature. <p><i>No or insufficient amounts of antibiotic used during cultivation</i></p> <ul style="list-style-type: none"> • Cells carrying the plasmid of interest may become overgrown by non-transformed cells, when inadequate levels of the appropriate antibiotics are used. Add appropriate amounts of freshly prepared stock solutions to all media; both solid and liquid. <p><i>Bacterial culture too old</i></p> <ul style="list-style-type: none"> • Do not incubate cultures for more than 16 h at 37 °C under shaking. We recommend LB as the optimal growth medium; however, when using very rich media like TB (terrific broth), cultivation time should be reduced to < 12 h. <p><i>Suboptimal elution conditions</i></p> <ul style="list-style-type: none"> • If possible, use a slightly alkaline elution buffer like Buffer AE (5 M Tris/HCl, pH 8.5). If nuclease-free water is used, check the pH of the water. Elution efficiencies drop drastically with buffers < pH 7.

Problem	Possible cause and suggestions
Poor plasmid yield (continued)	<p data-bbox="317 208 727 235"><i>No high copy number plasmid was used</i></p> <ul data-bbox="317 248 962 353" style="list-style-type: none"> • For NucleoSpin® Plasmid/Plasmid (NoLid): If using low copy number plasmids (e.g., plasmids bearing the P15A ori, cosmids, or P1 constructs), the culture volumes should be increased to at least 5 mL.
No plasmid yield	<p data-bbox="317 376 628 403"><i>Reagents not applied properly</i></p> <ul data-bbox="317 416 962 472" style="list-style-type: none"> • Add indicated volume of 96–100% ethanol to Buffer A4 and Buffer AQ Concentrate and mix thoroughly (see section 3). <p data-bbox="317 486 639 512"><i>Nuclease-rich host strains used</i></p> <ul data-bbox="317 526 962 607" style="list-style-type: none"> • Especially when working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid DNA degradation. <ul data-bbox="317 620 962 790" style="list-style-type: none"> • For NucleoSpin® Plasmid/Plasmid (NoLid): If using nuclease-rich strains like <i>E. coli</i> HB101 or strains of the JM series, be sure to perform the optional AW washing step (step 5; section 5.1). Optimal endonuclease removal can be achieved by incubating the membrane with preheated Buffer AW (50 °C) for 2 min before centrifugation. <p data-bbox="317 804 703 831"><i>Inappropriate storage of plasmid DNA</i></p> <ul data-bbox="317 844 962 954" style="list-style-type: none"> • Quantitate DNA directly after preparation, for example, by agarose gel electrophoresis. Store plasmid DNA dissolved in water at < -18 °C or at < +5 °C when dissolved in Buffer AE or TE buffer.
Poor plasmid quality	<p data-bbox="317 976 528 1003"><i>Nicked plasmid DNA</i></p> <ul data-bbox="317 1016 962 1072" style="list-style-type: none"> • Cell suspension was incubated with alkaline Lysis Buffer A2 for more than 5 min. <p data-bbox="317 1086 616 1113"><i>Genomic DNA contamination</i></p> <ul data-bbox="317 1126 962 1184" style="list-style-type: none"> • Cell lysate was vortexed or mixed too vigorously after addition of Buffer A2. Genomic DNA was sheared and thus liberated.

Problem	Possible cause and suggestions
Poor plasmid quality (continued)	<i>Smearred plasmid bands on agarose gel</i>
	<ul style="list-style-type: none"> Especially when working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid DNA degradation. For NucleoSpin® Plasmid/Plasmid (NoLid): If using nuclease-rich strains like <i>E. coli</i> HB101 or strains of the JM series, be sure to perform the optional AW washing step (step 5; section 5.1). Optimal endonuclease removal can be achieved by incubating the membrane with preheated Buffer AW (50 °C) for 2 min before centrifugation.
Suboptimal performance of plasmid DNA in enzymatic reactions	<i>Carry-over of ethanol</i>
	<ul style="list-style-type: none"> For NucleoSpin® Plasmid/Plasmid (NoLid): Make sure to centrifuge ≥ 1 min at 11,000 x <i>g</i> in step 6 in order to achieve complete removal of ethanolic Buffer A4. For NucleoSpin® Plasmid QuickPure: Make sure to centrifuge ≥ 3 min at 11,000 x <i>g</i> in step 5 in order to achieve complete removal of ethanolic Buffer AQ.
	<i>Elution of plasmid DNA with TE buffer</i>
	<ul style="list-style-type: none"> EDTA may inhibit sequencing reactions. Repurify plasmid DNA and elute with Buffer AE or water. Alternatively, the eluted plasmid DNA can be precipitated with ethanol and redissolved in Buffer AE or water.
	<i>No additional washing with Buffer AW performed</i>
	<ul style="list-style-type: none"> For NucleoSpin® Plasmid/Plasmid (NoLid): Additional washing with 500 μL Buffer AW before washing with ethanolic Buffer A4 will increase the reading length of sequencing reactions.
	<i>Not enough DNA used for sequencing reaction</i>
	<ul style="list-style-type: none"> Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions.
	<i>Plasmid DNA prepared from too much bacterial cell material</i>
	<ul style="list-style-type: none"> Do not use more than 3 mL of a saturated <i>E. coli</i> culture if preparing plasmid DNA for automated fluorescent DNA sequencing.

8.2 Ordering information

Product	REF	Pack of
NucleoSpin® Plasmid	740588.10/.50/.250	10/50/250 preps
NucleoSpin® Plasmid (NoLid)	740499.10/.50/.250	10/50/250 preps
NucleoSpin® Plasmid EasyPure	740727.10/.50/.250	10/50/250 preps
NucleoSpin® Plasmid QuickPure	740615.10/.50/.250	10/50/250 preps
NucleoSpin® Buffer Set (for the isolation of low-copy plasmids)	740953	1
Buffer A1 (without RNase A)	740911.1	1 L
Buffer A2 (without LyseControl)	740912.1	1 L
Buffer A3	740913.1	1 L
Buffer A4 (Concentrate) (for 125 mL Buffer A4)	740914	25 mL
Buffer A4 (Concentrate) (for 1 L Buffer A4)	740914.1	200 mL
Buffer AW	740916.1	1 L
Buffer AE	740917.1	1 L
RNase A (lyophilized)	740505 740505.50	100 mg 50 mg
Collection Tubes (2 mL)	740600	1000

8.3 References

Birnboim, H.C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening of recombinant plasmid DNA. *Nucleic Acids Res.* **7**: 1513-1523.

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA* **76**: 615-619.

8.4 Product use restriction/warranty

NucleoSpin® Plasmid/Plasmid (NoLid) and **NucleoSpin® Plasmid QuickPure** 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 24 21 969-270
tech-bio@mn-net.com

Trademarks:

NucleoSpin® is a registered trademark of MACHEREY-NAGEL GmbH & Co. KG

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.



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

A023774 / 03910