Plasmid DNA Purification

User Manual

NucleoSpin® Plasmid

NucleoSpin® Plasmid (NoLid)

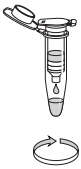
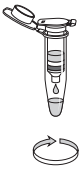
NucleoSpin® Plasmid QuickPure

July 2010 / Rev. 07

MACHEREY-NAGEL

Plasmid DNA Purification

Protocol-at-a-glance (Rev. 07)

		NucleoSpin® Plasmid	NucleoSpin® Plasmid (NoLid)		NucleoSpin® Plasmid QuickPure	
1	**Cultivate and harvest bacterial cells**			11,000 x *g* 30 s		11,000 x *g* 30 s
2	**Cell lysis**			250 µL Buffer A1 250 µL Buffer A2 RT, 5 min 300 µL Buffer A3		250 µL Buffer A1 250 µL Buffer A2 RT, 5 min 300 µL Buffer A3
3	**Clarification of the lysate**			11,000 x *g* 5 – 10 min		11,000 x *g* 5 min
4	**Bind DNA**			Load supernatant 11,000 x *g* 1 min		Load supernatant 11,000 x *g* 1 min
5	**Wash silica membrane**			*(Optional: 500 µL Buffer AW)* 600 µL Buffer A4 11,000 x *g* 1 min		450 µL Buffer AQ 11,000 x *g* 3 min
6	**Dry silica membrane**			11,000 x *g* 2 min		Drying is perfomed during centrifugation of the single washing step
7	**Elute DNA**			50 µL Buffer AE RT, 1 min 11,000 x *g* 1 min		50 µL Buffer AE RT, 1 min 11,000 x *g* 1 min

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1 Components

1.1 Kit contents

	NucleoSpin® Plasmid		
	10 preps	**50 preps**	**250 preps**
REF	**740588.10**	**740588.50**	**740588.250**
Resuspension Buffer A1	5 mL	15 mL	75 mL
Lysis Buffer A2	5 mL	15 mL	3 x 25 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)*	2 mL	2 x 6 mL	2 x 20 mL
Elution Buffer AE**	5 mL	15 mL	75 mL
RNase A (lyophilized)*	2 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

1.1 Kit contents *continued*

	NucleoSpin® Plasmid (NoLid)		
	10 preps	**50 preps**	**250 preps**
REF	**740499.10**	**740499.50**	**740499.250**
Resuspension Buffer A1	5 mL	15 mL	75 mL
Lysis Buffer A2	5 mL	15 mL	3 x 25 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)*	2 mL	2 x 6 mL	2 x 20 mL
Elution Buffer AE**	5 mL	15 mL	75 mL
RNase A (lyophilized)*	2 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

1.1 Kit contents *continued*

	NucleoSpin® Plasmid QuickPure		
	10 preps	**50 preps**	**250 preps**
REF	**740615.10**	**740615.50**	**740615.250**
Resuspension Buffer A1	5 mL	15 mL	75 mL
Lysis Buffer A2	5 mL	15 mL	3 x 25 mL
Neutralization Buffer A3	5 mL	20 mL	100 mL
Wash Buffer AQ (Concentrate)*	2 mL	6 mL	2 x 20 mL
Elution Buffer AE**	5 mL	15 mL	75 mL
RNase A (lyophilized)*	2 mg	6 mg	30 mg
NucleoSpin® Plasmid QuickPure Columns (orange 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
- Manual pipettors and disposable pipette tips

Equipment

- 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 reading the detailed protocol sections of this User Manual if the **NucleoSpin® Plasmid / Plasmid (NoLid)** or **NucleoSpin® Plasmid QuickPure** 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 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.

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 5 – 10 mL low copy	1 – 3 mL high copy
Typical yield	< 25 µg (1 – 5 mL culture) < 40 µg (5 – 10 mL culture)	< 15 µg (1 – 3 mL culture)
Elution volume	50 µL	50 µL
Binding capacity	60 µg	15 µg
Vectors	< 15 kbp	< 15 kbp
Preparation time*	25 min/18 preps	11 min/18 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.

* Hands-on-time

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 goes down regardless of the cell mass. Table 2 gives information on concentrations of commonly used antibiotics.

Table 2: Information about antibiotics according to Maniatis*

Antibiotic	Stock solution (concentration)	Storage	Working concentration
Ampicillin	50 mg/mL in H_2O	-20°C	20 – 50 µg/mL
Carbenicillin	50 mg/mL in H_2O	-20°C	20 – 60 µg/mL
Chloramphenicol	34 mg/mL in EtOH	-20°C	25 – 170 µg/mL
Kanamycin	10 mg/mL in H_2O	-20°C	10 – 50 µg/mL
Streptomycin	10 mg/mL in H_2O	-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 4 to choose the best culture volume according to the optical density at 600 nm (OD_{600}).

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

OD_{600}	1	2	**3**	4	5	6
Culture volume	15 mL	8 mL	**5 mL**	4 mL	3 mL	2 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

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

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).

2.4 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 CO_2 leads to a decrease in pH of unbuffered solutions.

3 Storage conditions and preparation of working solutions

Attention:

Buffers A3 and AW contain guanidinium hydrochloride! Wear gloves and goggles!

- All kit components can be stored at room temperature (18 – 25°C) and are stable up to 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:

- Before the first use of the kit, add 1 mL of Buffer A1 to the RNase A vial and vortex. Transfer all of the resulting solution 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	**50 preps**	**250 preps**
REF	**740588.10 / 740499.10**	**740588.50 / 740499.50**	**740588.250 / 740499.250**
Wash Buffer A4 (Concentrate)	2 mL Add 8 mL ethanol	2 x 6 mL Add 24 mL ethanol to each bottle	2 x 20 mL Add 80 mL ethanol to each bottle

	NucleoSpin® Plasmid QuickPure		
	10 preps	**50 preps**	**250 preps**
REF	**740615.10**	**740615.50**	**740615.250**
Wash Buffer AQ (Concentrate)	2 mL Add 8 mL ethanol	6 mL Add 24 mL ethanol	2 x 20 mL Add 80 mL ethanol to each bottle

4 Safety instructions – risk and safety phrases

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

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

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
A2	Sodium hydroxide <2%	Xi*	Irritating to eyes and skin	R 36/38	S 26-37/39-45
A3	Guanidine hydrochloride	Xn**	Harmful if swallowed - Irritating to eyes and skin	R 22-36/38	
AW	Guanidine hydrochloride + isopropanol <25%	Xn**	Flammable - Harmful if swallowed - Irritating to eyes and skin.	R 10-22-36/38	S 7-16-25
RNase A	RNase A, lyophilized	Xi*	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24

Risk phrases

R 10	Flammable	R 36/38	Irritating to eyes and skin
R 22	Harmful if swallowed	R 42/43	May cause sensitization by inhalation and skin contact

Safety phrases

S 7	Keep container tightly closed
S 16	Keep away from sources of ignition - No smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 25	Avoid contact with the eyes
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 37/39	Wear suitable protective clothing and gloves
S 45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

* Hazard labeling not neccessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

** Hazard labeling not neccessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 Protocols for plasmid DNA preparation

5.1 NucleoSpin® Plasmid / Plasmid (NoLid): 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.

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

11,000 x *g*
30 s

2 Cell lysis

+ 250 µL A1

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!

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. Cool buffer down to room temperature (18 – 25°C).

+ 250 µL A2

Mix

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.

RT
5 min

+ 300 µL A3

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

Mix

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 750 µL of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through and place the NucleoSpin® Plasmid / Plasmid (NoLid) Column back into the collection tube.

Load supernatant

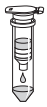
11,000 x *g*
1 min

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 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)

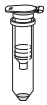
+ 600 µL A4

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

11,000 x *g*
1 min

6 Dry silica membrane

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

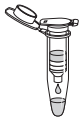


Note: Residual ethanolic wash buffer might inhibit enzymatic reactions.

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 2.4.

5.2 Support protocol for NucleoSpin® Plasmid / Plasmid (NoLid): 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).

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

+ 500 µL A1

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!

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. Cool buffer down to room temperature (18 – 25°C).

+ 500 µL A2

Mix

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.

RT
5 min

+ 600 µL A3

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

Mix

3 Clarification of lysate

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

11,000 x *g*
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 750 µL of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through 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

(Optional: + 500 µL AW

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

***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 flow-through 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.

<u>Note</u>: 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

70°C
2 min

<u>Note</u>: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see section 2.4.

11,000 x *g*
1 min

5.3 NucleoSpin® Plasmid QuickPure: 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

+ 250 µL A1

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!

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. Cool buffer down to room temperature (18 – 25°C).

+ 250 µL A2

Mix

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.

RT
5 min

+ 300 µL A3

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

Mix

3 Clarification of lysate

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

11,000 x *g*
5 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 750 µL of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through 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 flow-through 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

11,000 x *g*
1 min

<u>Note</u>: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see section 2.4.

5.4 Support protocol for NucleoSpin® Plasmid / Plasmid (NoLid) and NucleoSpin® Plasmid QuickPure: 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

+ 250 µL A1
+ Lysozyme

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!

Resuspend

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

37°C
10 – 30 min

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 5.3).

5.5 Support protocol for NucleoSpin® Plasmid / Plasmid (NoLid) and NucleoSpin® Plasmid QuickPure: 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.

(E.g., add 200 µL Buffer A3 to 100 µL enzymatic reaction mix.)

+ 2 vol A3

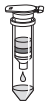
Mix

2 Bind DNA

Place a NucleoSpin® Plasmid / Plasmid (NoLid) or 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 flow-through and place the NucleoSpin® Plasmid / Plasmid (NoLid) Column or Plasmid QuickPure Column back into the collection tube.

Note: Maximum loading capacity of the NucleoSpin® Plasmid / Plasmid (NoLid) Column or Plasmid QuickPure Column is 750 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 5.3).



Load mixture

11,000 x *g*
1 min

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of bacterial cells	*Cell pellet not properly resuspended* • It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of Buffer A2. *SDS in Buffer A2 precipitated* • 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. *Too many bacterial cells used* • 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.
Poor plasmid yield	*Incomplete lysis of bacterial cells* • See „Possible cause and suggestions“ above. *Suboptimal precipitation of SDS and cell debris* • Precipitation of SDS and cell debris will be slightly more effective when centrifuging at 4°C instead of room temperature. *No or insufficient amounts of antibiotic used during cultivation* • 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. *Bacterial culture too old* • 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.

Poor plasmid yield *(continued)*	*Suboptimal elution conditions* • 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. *No high copy-number plasmid was used* • **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	*Reagents not applied properly* • Add indicated volume of 96 – 100% ethanol to Buffer A4 and Buffer AQ Concentrate and mix thoroughly (see section 3). *Nuclease-rich host strains used* • 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 *E. coli* 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. *Inappropriate storage of plasmid DNA* • 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	*Nicked plasmid DNA* • Cell suspension was incubated with alkaline Lysis Buffer A2 for more than 5 min. *Genomic DNA contamination* • Cell lysate was vortexed or mixed too vigorously after addition of Buffer A2. Genomic DNA was sheared and thus liberated.

Poor plasmid quality *(continued)*	*Smeared plasmid bands on agarose gel* • 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 *E. coli* 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	*Carry-over of ethanol* • **For NucleoSpin® Plasmid / Plasmid (NoLid)**: Make sure to centrifuge ≥ 1 min at 11,000 x *g* 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 *g* in step 5 in order to achieve complete removal of ethanolic Buffer AQ. *Elution of plasmid DNA with TE buffer* • 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. *No additional washing with Buffer AW performed* • **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. *Not enough DNA used for sequencing reaction* • Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions. *Plasmid DNA prepared from too much bacterial cell material* • Do not use more than 3 mL of a saturated *E. coli* culture if preparing plasmid DNA for automated fluorescent DNA sequencing.

6.2 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 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	740912.1	1 L
Buffer A3	740913.1	1 L
Buffer A4 Concentrate (for 100 mL Buffer A4)	740914	20 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	740505	100 mg
RNase A	740505.50	50 mg
Collection Tubes (2 mL)	740600	1000

6.3 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.

6.4 Product use restriction / warranty

NucleoSpin® Plasmid / Plasmid (NoLid) and **NucleoSpin® Plasmid QuickPure** kit components were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY.** They are suitable **FOR *IN-VITRO* USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoSpin® Plasmid / Plasmid (NoLid)** and **NucleoSpin® Plasmid QuickPure** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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Last updated: 12/2006, Rev. 02