Bioanalysis

MACHEREY-NAGEL

User manual



Plasmid DNA purification

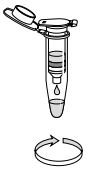
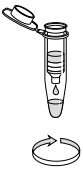
- NucleoSpin® Plasmid
- NucleoSpin® Plasmid (NoLid)

November 2024 / Rev. 15

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Plasmid DNA purification

Protocol at a glance (Rev. 15)

	NucleoSpin® Plasmid	NucleoSpin® Plasmid (NoLid)	
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			*(Optional: 500 µL Buffer AW: RT or 50 °C)* 600 µL Buffer A4 11,000 x *g*, 1 min
6 Dry silica membrane			11,000 x *g*, 2 min
7 Elute DNA			50 µL Buffer AE RT, 1 min 11,000 x *g*, 1 min

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MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-333 · support@mn-net.com · *www.mn-net.com*

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1 Components

1.1 Kit contents

	NucleoSpin® Plasmid		
REF	**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 × 75 mL
Wash Buffer A4 (Concentrate)*	6 mL	12 mL	2 × 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

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* 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 (NoLid)		
REF	**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 × 75 mL
Wash Buffer A4 (Concentrate)*	6 mL	12 mL	2 × 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

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* 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)** 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.

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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) 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) 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.

2.2 Kit specifications

- The **NucleoSpin® Plasmid / Plasmid (NoLid)** kit is 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 plasmid DNA prepared with **NucleoSpin® Plasmid / Plasmid (NoLid)** is suitable for applications like automated fluorescent DNA sequencing, PCR, or any kind of enzymatic manipulation.

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- 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)
Use	For research use only
Culture volume	1 – 5 mL (high copy) 6 – 10 mL (low copy)
Typical yield	< 25 µg (1 – 5 mL culture) < 45 µg (6 – 10 mL culture)
Elution volume	50 µL
Binding capacity	60 µg
Vectors	< 25 kbp
Preparation time*	20 min/6 preps
Format	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

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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)** 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 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 (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

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 Lysate neutralization and LyseControl

Proper mixing of the lysate with Neutralization Buffer A3 is of most 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.

* 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.

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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 CO_2 leads to a decrease in pH of unbuffered solutions.

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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 15 – 25 °C and are stable until: see package label.
- 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)** 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.

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

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4 Safety instructions

When working with the **NucleoSpin® Plasmid/Plasmid (NoLid)** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult theappropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

CAUTION: Guanidine hydrochloride in buffer A3 and buffer AW can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® Plasmid/Plasmid (NoLid)** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should behandled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

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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 × 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 × g, 5 – 10 min

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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 × g, 1 min

Repeat this step to load the remaining lysate.

5 Wash silica membrane

<u>Recommended:</u> 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 × 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 × 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 × 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 × *g***.



+ 50 µL AE

RT, 1 min



11,000 × g, 1 min

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

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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 6.2).

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 × 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 × g, 5 – 10 min

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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 × 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 × 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 × g, 1 min

6 Dry silica membrane

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





11,000 × g, 2 min

Note: Residual ethanolic wash buffer might inhibit enzymatic reactions.

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 × *g***.



+ 50 µL AE

RT, 1 min



11,000 × g, 1 min

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

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5.3 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 was prepared according to section 3.

1 Cultivate and harvest bacterial cells

Use up to **5 mL** (NucleoSpin® Plasmid / Plasmid (NoLid)) 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 × 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 5.5).

37 °C, 10 – 30 min

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5.4 Isolation of high-copy plasmid DNA using a vacuum manifold

1 Cultivate and harvest bacterial cells



1 – 5 mL *E. coli* culture

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



11,000 × g, 30 s

Note: For isolation of low-copy plasmids volumes of culture and buffers need to be adapted. Refer to section 5.2 in the NucleoSpin® Plasmid / Plasmid (NoLid) user manual.

2 Lyse cells

+ 250 µL A1

Resuspend

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!

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 any precipitate is dissolved. 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 a maximum of **5 min** or until the lysate appears clear.

+ 350 µL A3

Mix

Add **350 µL Buffer A3**. **Mix thoroughly** by inverting the tube **until the blue samples turn colorless completely**!

Do not vortex to avoid shearing of genomic DNA!

3 Clarify lysate



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



11,000 × g, 10 min

Repeat this step in case the supernatant is not clear!

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4 Bind DNA

Place a **NucleoSpin® Plasmid / Plasmid (NoLid) Column** onto a suitable vacuum manifold with Luerconnections like the NucleoVac 24 Vacuum Manifold and **load up to 700 µL supernatant**. Do not close the lid!



Load supernatant

Apply vacuum of **-0.2 to -0.4 bar* (1 min)**.

When the sample has passed the NucleoSpin® Plasmid / Plasmid (NoLid) Column, release the vacuum.

-0.2 to -0.4 bar*, 1 min

If necessary, load remaining sample and repeat the step.

5 Wash silica membrane

(*Optional: Add **500 µL Buffer AW**, optionally preheated to 50 °C, to remove protein.*)



(Optional: + 500 µL AW -0.2 to -0.4 bar*, 1 min)

Apply vacuum of **-0.2 to -0.4 bar* (1 min)**. When the buffer has passed the NucleoSpin® Plasmid / Plasmid (NoLid) Column, release the vacuum.

+ 600 µL A4

Add **600 µL Buffer A4** (supplemented with ethanol, see section 3). Apply vacuum of **-0.2 to -0.4 bar* (1 min)**.

-0.2 to -0.4 bar*, 1 min

When the buffer has passed the NucleoSpin® Plasmid / Plasmid (NoLid) Column, release the vacuum.

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* Reduction of atmospheric pressure

6 Dry silica membrane



-0.2 to -0.4 bar*, 5 min

Option 1: Drying by vacuum

Apply vacuum of **-0.4 to -0.6 bar*** for **5 min** to remove Buffer A4 completely. Run the vacuum pump continuously. Achieving and keeping a continuous air flow is more important than reaching the exact mentioned reduction of atmospheric pressure. Do not close the lid!

Release the vacuum.

Option 2: Drying by centrifugation



Place the NucleoSpin® Plasmid / Plasmid (NoLid) Column into a Collection Tube (2 mL). Centrifuge for **1 min** at **11,000 × *g*** to remove Buffer A4 completely. Make sure the spin column does not come in contact with the flowthrough while removing it from the centrifuge and the collection tube.



11,000 × g, 1 min

Note: Residual ethanol from Buffer A4 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 min at 70 °C prior to elution.

7 Elute DNA



+ 50 µL AE

RT, 1 min

Place the NucleoSpin® Plasmid / Plasmid (NoLid) Column into a new 1.5 mL microcentrifuge tube (not provided).

Add **50 µL Buffer AE** and incubate at **room temperature** (18 – 25 °C) for **1 min**. Centrifuge for **1 min** at **11,000 × *g***.



11,000 × g, 1 min

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* Reduction of atmospheric pressure

5.5 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) by omitting the cell lysis step.

Before starting the preparation:

- Check if Wash Buffer A4 was 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) 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 back into the collection tube.

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



Load mixture

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).



11,000 × g, 1 min

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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. *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.

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Problem	Possible cause and suggestions
Poor plasmid yield *(continued)*	*No high copy number plasmid was used* • 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 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. • 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. • 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.

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Problem	Possible cause and suggestions
Suboptimal performance of plasmid DNA in enzymatic reactions	*Carry-over of ethanol* • Make sure to centrifuge ≥ 1 min at 11,000 × *g* in step 6 in order to achieve complete removal of ethanolic Buffer A4. *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* • 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.

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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 EasyPure	740727.10/.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

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.

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6.4 Product use restriction / warranty

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support@mn-net.com

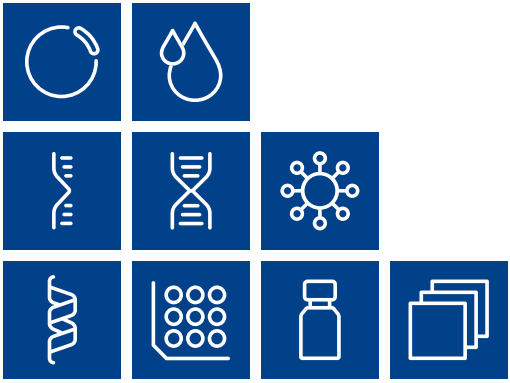
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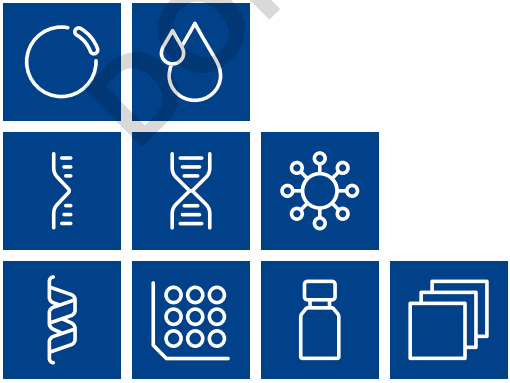
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MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany
DE +49 24 21 969-0 info@mn-net.com
FR +33 388 68 22 68 sales-fr@mn-net.com
CH +41 62 388 55 00 sales-ch@mn-net.com
US +1 888 321 62 24 sales-us@mn-net.com