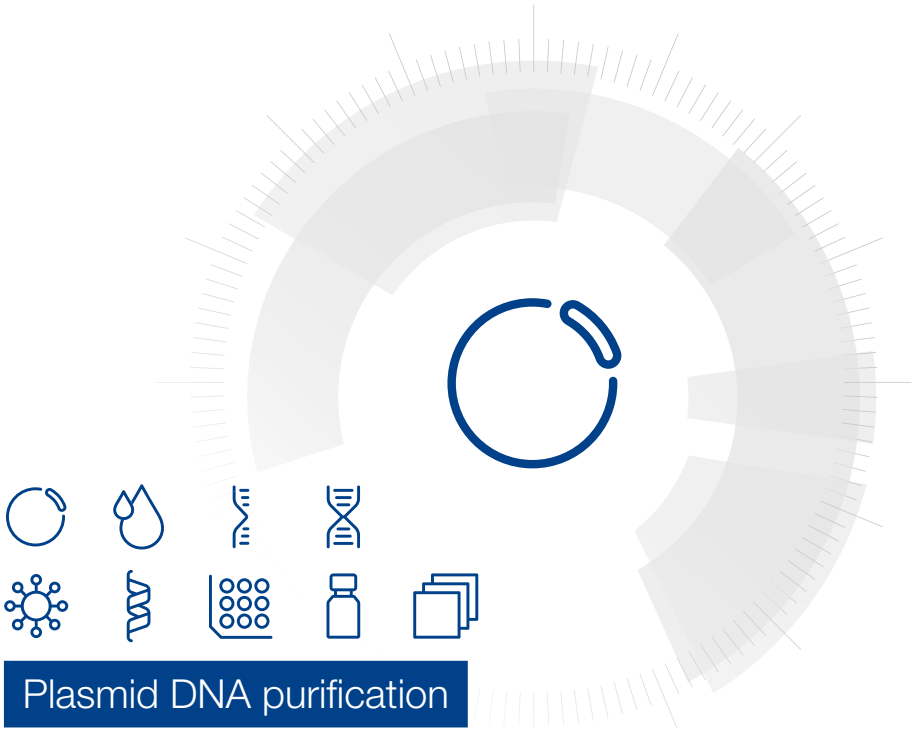
Bioanalysis

MACHEREY-NAGEL

User manual



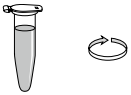
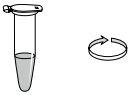
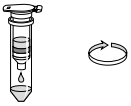
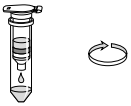
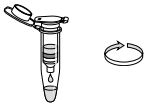
Plasmid DNA purification

- NucleoSpin® Plasmid EasyPure

November 2025 / Rev. 05

Plasmid DNA purification

Protocol at a glance (Rev. 05)

	NucleoSpin® Plasmid EasyPure
1 Cultivate and harvest bacterial cells	 12,000 × *g*, 30 s
2 Cell lysis	 150 µL Buffer A1 250 µL Buffer A2 RT, up to 2 min 350 µL Buffer A3
3 Clarification of the lysate	 > 12,000 × *g*, 3 min
4 Bind DNA	 Load supernatant 1,000 – 2,000 × *g*, 30 s
5 Wash and dry silica membrane	 450 µL Buffer AQ > 12,000 x *g*, 1 min
6 Elute DNA	 50 µL Buffer AE RT, 1 min > 12,000 × *g*, 1 min

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1 Components

1.1 Kit contents

	NucleoSpin® Plasmid EasyPure		
REF	**10 preps 740727.10**	**50 preps 740727.50**	**250 preps 740727.250**
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 AQ (Concentrate)*	6 mL	6 mL	25 mL
Elution Buffer AE**	13 mL	13 mL	30 mL
Liquid RNase A***	2 mg	6 mg	30 mg
NucleoSpin® Plasmid EasyPure Columns (dark blue rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
Short protocol	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

*** Amount of RNase A (in mg) dissolved in respective volume of buffer

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
- 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 EasyPure** 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.

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

2 Product description

2.1 Basic principle

With the **NucleoSpin® Plasmid EasyPure** 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 EasyPure Column**. Precipitated protein, genomic DNA, and cell debris are then pelleted by a centrifugation step. The supernatant is loaded onto a **NucleoSpin® Plasmid EasyPure Column**.

With the **NucleoSpin® Plasmid EasyPure** 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 EasyPure** kits are designed for the rapid, small-scale preparation of highly pure plasmid DNA (mini preps).
- The **NucleoSpin® Plasmid EasyPure Column** features a new specially treated silica membrane which allows speeding up the procedure by a combined washing and drying step. The number of washing and drying steps is reduced from 3 to only 1!
- LyseControl: The Lysis Buffer A2 contains a blue pH indicator to ensure complete neutralization for maximum yield.
- The purified plasmid DNA is suitable for applications like automated fluorescent DNA sequencing, PCR, or any kind of enzymatic manipulation.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Plasmid EasyPure
Culture volume	2 – 10 mL
Typical yield	15 – 30 µg
Elution volume	50 µL
Binding capacity	35 µg
Vectors	< 15 kpb
Preparation time*	14 min/6 preps
Format	Mini spin column
Use	For research use only

* Hands-on-time

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 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 LB culture 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}).

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

Table 3: Recommended culture volumes for NucleoSpin® Plasmid EasyPure						
OD_{600}	1	2	**3**	4	5	6
Culture volume	15 mL	8 mL	**5 mL**	4 mL	3 mL	2 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 increase all lysis buffers proportionally.

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 EasyPure 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: Buffer A3 contains guanidine hydrochloride! Wear gloves and goggles!

CAUTION: Buffer A3 contains 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 (15 – 25 °C) and are stable for at least one year.
- Always keep buffer bottles tightly closed, especially if buffers are preheated during the preparation.
- Storage of Buffer A2 below 20 °C may cause precipitation of SDS. If salt precipitate is observed, incubate buffer at 30 – 40 °C for several minutes and mix well until all precipitate is redissolved completely. Cool down to room temperature before use.

Before starting any **NucleoSpin® Plasmid EasyPure** protocol prepare the following:

- Add Liquid RNase A to Buffer A1 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 AQ (Concentrate).

	NucleoSpin® Plasmid EasyPure		
REF	**10 preps** **740727.10**	**50 preps** **740727.50**	**250 preps** **740727.250**
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

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

Caution: Guanidine hydrochloride in buffer A3, sodium hydroxide in buffer A2 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 EasyPure** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

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

5 NucleoSpin® Plasmid EasyPure protocol

Before starting the preparation:

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

1 Cultivate and harvest bacterial cells

Use **2 – 10 mL** of a saturated ***E. coli* culture** (see page 8, Table 3) pellet cells in a standard benchtop microcentrifuge for **30 s** at **> 12,000 × *g***.

Discard the supernatant and remove as much of the liquid as possible.

> 12,000 × *g*, 30 s

2 Cell lysis

Add **150 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!

+ 150 µL A1

Resuspend

Attention: Check Buffer A2 for precipitated SDS. 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 (15 – 25 °C) before use.

+ 250 µL A2

Mix

TA, 2 min

Add **250 µL Buffer A2**. Mix gently by inverting the tube **5 times**. Do not vortex to avoid shearing of genomic DNA. Incubate at **room temperature (15 – 25 °C)** for up to **2 min** or until lysate appears clear.

+ 350 µL A3

Mix

Add **350 µL Buffer A3**. Mix thoroughly by inverting the tube until LyseControl has turned **colorless** throughout the lysate without any traces of blue color. Do not vortex to avoid shearing of genomic DNA!

3 Clarification of lysate

Centrifuge for **3 min** at **full speed** (**> 12,000 × *g***).

> 12,000 × *g*, 3 min

4 Bind DNA

Place a NucleoSpin® Plasmid EasyPure Column into a Collection Tube (2 mL) and decant the supernatant from step 3 onto the column.

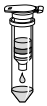
Load supernatant

Centrifuge for **30 s** at **1,000 – 2,000 × *g***.

1,000 – 2,000 × g, 30 s

Discard flow-through and place the spin column back into the collection tube.

5 Wash and dry silica membrane



+ 450 µL AQ

Add **450 µL Buffer AQ** (supplemented with ethanol, see section 3).

Centrifuge for **1 min** at **full speed** (> 12,000 × *g*).

> 12,000 × *g*, 1 min

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.

Note: To reduce ethanol carry-over to a minimum for better performance in downstream applications, incubate spin cup for 10 – 15 min at 37 °C to dry silica membrane completely.

6 Elute DNA

+ 50 µL AE

RT, 1 min

Place the NucleoSpin® Plasmid EasyPure Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL Buffer AE**.

Incubate for **1 min** at **room temperature (15 – 25 °C)**.

> 12,000 x *g*, 1 min

Centrifuge for **1 min** at **full speed** (> 12,000 x *g*).

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

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* • Storage of Buffer A2 below 20 °C may cause precipitation of SDS. If salt precipitate is observed, incubate buffer at 30 – 40 °C for several minutes and mix well until all precipitate is redissolved completely. Cool down to room temperature before use. *Too many bacterial cells used* • See table 3 for maximum amount of cells.
Poor plasmid yield	*Incomplete lysis of bacterial cells* • See „Possible cause and suggestions“ above. *No or insufficient amounts of antibiotic used during cultivation* • Cells carrying the plasmid of interest may become overgrown by cells without plasmid (see table 2), when inadequate levels of the 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 (LB) or 12 h (rich media) at 37 °C under shaking to avoid starvation and plasmid degradation. *Incomplete neutralization* • Mix thoroughly after addition of Buffer A3 until LyseControl has turned colorless without any traces of blue. *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. *Low copy-number plasmid was used* • At least double or triple culture volume and increase lysis buffers if final amount of cells exceed the recommended volumes of table 3.

Problem	Possible cause and suggestions
No plasmid yield	*Reagents not applied properly* • Add indicated volume of 96 – 100 % ethanol to 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. *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 too long. Reduce lysis time. *Genomic DNA contamination* • Cell lysate was vortexed or mixed too vigorously after addition of Buffer A2. Genomic DNA was sheared and thus liberated. *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.
Suboptimal performance of plasmid DNA in enzymatic reactions	*Carry-over of ethanol* • Make sure that the NucleoSpin® Plasmid EasyPure Column is completely dry after step 5. Otherwise discard flow-through and repeat centrifugation. *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. *Not enough DNA used for sequencing reaction* • Quantify DNA by agarose gel electrophoresis before setting up sequencing reactions.

6.2 Ordering information

Product	REF	Pack of
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	740912.1	1 L
Buffer A3	740913.1	1 L
Buffer AQ (Concentrate) (for 125 mL Buffer AQ)	740995	25 mL
Buffer AE	740917.1	1 L
Liquid RNase A	740397	250 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

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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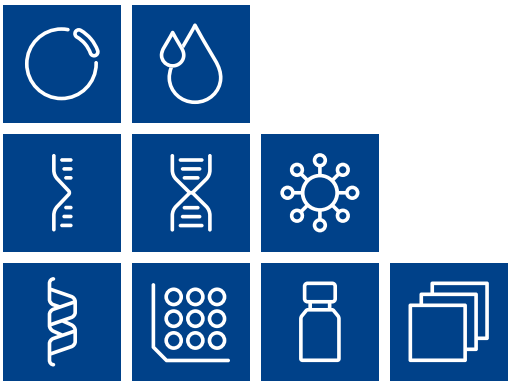
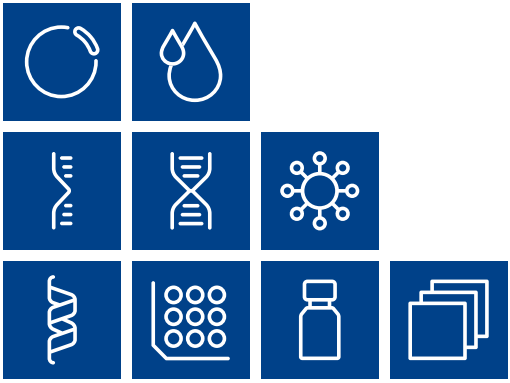


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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