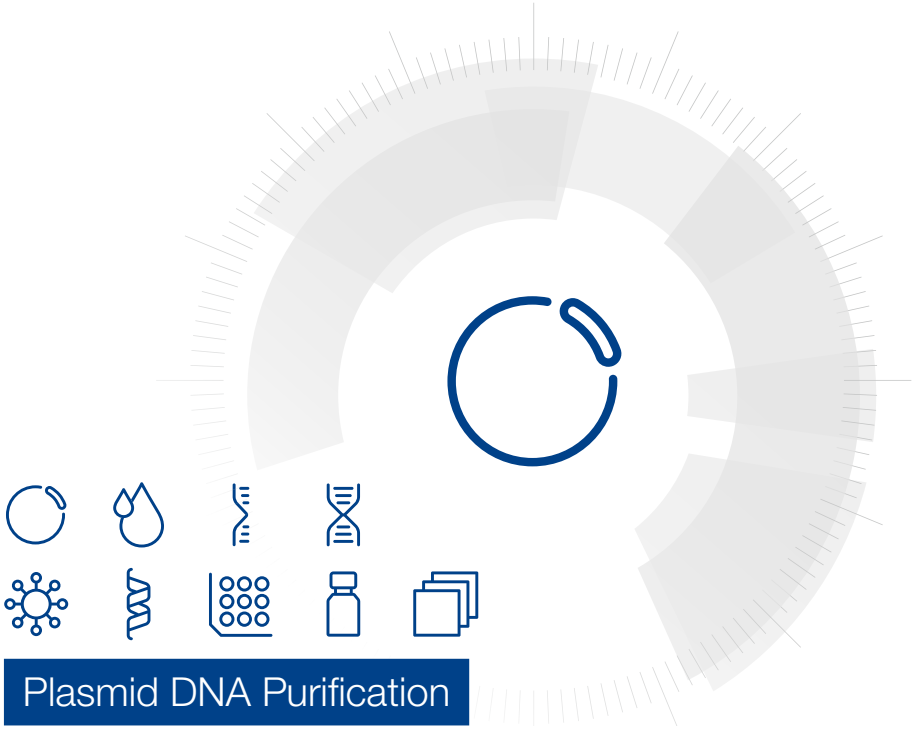
Bioanalysis

MACHEREY-NAGEL

User manual



Plasmid DNA Purification

■ NucleoSpin® Plasmid Transfection-grade Plus

October 2026 / Rev. 04

Contact MN

Germany and international

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-0
Toll-free: 0800 26 16 000 (Germany only)
E-mail: info@mn-net.com

Technical Support Bioanalysis

Tel.: +49 24 21 969-333
E-mail: support@mn-net.com

USA

MACHEREY-NAGEL Inc.
924 Marcon Blvd. · Suite 102 · Allentown PA, 18109 · USA
Toll-free: 888 321 6224 (MACH)
E-mail: sales-us@mn-net.com

France

MACHEREY-NAGEL SAS
1, rue Gutenberg – BP135 · 67720 Hoerdt Cedex · France
Tel.: +33 388 68 22 68
E-mail: sales-fr@mn-net.com

MACHEREY-NAGEL SAS (Société par Actions Simplifiée) au capital de 186600 €
Siret 379 859 531 00020 · RCS Strasbourg B379859531 · N° intracommunautaire FR04 379 859 531

Switzerland

MACHEREY-NAGEL AG
Hirsackerstr. 7 · 4702 Oensingen · Switzerland
Tel.: +41 62 388 55 00
E-mail: sales-ch@mn-net.com

www.mn-net.com

Table of contents

1 Components 4
1.1 Kit contents 4
1.2 Reagents, consumables, and equipment to be supplied by user 5
1.3 About this user manual 5
2 Product description 6
2.1 Basic principle 6
2.2 Kit specifications 7
2.3 Growth of bacterial cultures 7
2.4 Scaling up for high cell mass 8
2.5 Elution procedures 8
3 Storage conditions and preparation of working solutions 9
4 Safety instructions 10
4.1 Disposal 10
5 Protocols 11
5.1 Isolation of transfection-grade plasmid DNA in single spin format 11
6. Appendix 13
6.1 Troubleshooting 13
6.2 Ordering information 15
6.3 Product use restriction / warranty 16

1 Components

1.1 Kit contents

	NucleoSpin® Plasmid Transfection-grade Plus		
REF	**10 preps 740500.10**	**50 preps 740500.50**	**250 preps 740500.250**
Resuspension Buffer A1	5 mL	15 mL	75 mL
Lysis Buffer A2	15 mL	15 mL	100 mL
Neutralization Buffer A3	5 mL	20 mL	100 mL
Detoxification Buffer ERB	13 mL	50 mL	200 mL
Wash Buffer AQ (Concentrate)*	6 mL	2 × 6 mL	2 × 25 mL
H_2O-EF	13 mL	13 mL	60 mL
RNase A (lyophilized)*	2.5 mg	6 mg	30 mg
NucleoSpin® Plasmid TG Plus Columns (orange rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
Leaflet	1	1	1

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

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
- Personal protection equipment (lab coat, gloves, goggles)
- **Required hardware:** A suitable centrifuge is required for harvesting the bacteria (either plate or tube centrifuge) and for the elution step under centrifugation.

1.3 About this user manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also 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 or updated revisions.

2 Product description

2.1 Basic principle

The **NucleoSpin® Plasmid Transfection-grade Plus** procedure is a modified version of the Birnboim and Doly[1] alkaline lysis plasmid miniprep protocol. Pelleted bacteria are resuspended in Buffer A1 and plasmid DNA is liberated from the cells by SDS/alkaline Lysis Buffer A2. Buffer A3 neutralizes the lysate, precipitates genomic DNA, proteins and cell debris, and creates appropriate conditions for binding of plasmid DNA to the silica membrane.

The crude lysate is cleared by centrifugation and brought into contact with a silica membrane where plasmid DNA binds to the surface. Endotoxins and proteins are removed by the Detoxification Buffer ERB. Further contaminations such as salts are removed with ethanolic Buffer AQ while traces of ethanol are removed by centrifugation.

Pure plasmid DNA is eluted in H_2O-EF and is ready for any common downstream application including transfection (research use only).

[1] Birnboim, H.C. & Doly, J. (1979) Nucleic Acids Res. 7, 1513 – 1523

2.2 Kit specifications

The **NucleoSpin® Plasmid Transfection-grade Plus** is designed for the rapid manual purification of transfection-grade plasmid DNA from *E. coli*.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Plasmid Transfection-grade Plus
Format	Mini spin columns
Processing	Manual, centrifugation or vacuum
Sample material	≤ 5 mL *E. coli* culture
Vector size	≤ 25 kbp
Elution volume	30 – 50 µL
Preparation time	25 min/18 preps
Theoretical binding capacity	100 µg
Typical yield	35 – 60 µg
Endotoxin level	≤ 1 EU/µg DNA
Use	For research use only

2.3 Growth of bacterial cultures

Plasmid yield and quality highly depend on the bacterial culture which is influenced by many factors.

Besides culture medium, selective antibiotic, bacterial host strain and type of plasmid, the oxygen availability is of crucial importance for bacterial growth. Anaerobic metabolism of nutrients will result in suboptimal energy uptake and accumulation of organic acids as metabolic end products which inhibit further cell growth.

The **NucleoSpin® Plasmid Transfection-grade Plus** kit is optimized for the purification of plasmid DNA from up to 5 mL bacterial culture with an OD_{600} of 3 or up to 3 mL of a culture with an OD_{600} of 5. Using significantly more bacteria will overload the lysis capacity and result in reduced yield. 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 cell mass shall be processed, see section 2.4.

2.4 Scaling up for high cell mass

When processing higher cell masses, either scale up buffer volumes proportionally before loading the column or split the lysis into two microcentrifuge tubes using standard buffer volumes and apply both cleared lysates to a single column. For extremely high cell masses, combining both methods is necessary to not exceed the capacity of the microcentrifuge tubes. Regardless of the method chosen, loading the **NucleoSpin® Plasmid Transfection-grade Plus** column must be restricted to a maximum of two loading steps before resuming the standard protocol (section 5) at step 5.

Example: For an 8 mL bacterial culture with an OD_{600} of 5, the lysis reaction must be split into two tubes, with each reaction requiring 340 µL of buffer A1, 340 µL of buffer A2 and 410 µL of buffer A3. Mixing of the buffers should be performed as stated in the standard protocol (section 5).

Note: We do NOT recommend going above 8 mL bacterial culture with an OD_{600} of 5.

2.5 Elution procedures

The elution buffer volume and method can be adapted to the subsequent downstream applications to maximize yield or concentration compared to the standard method.

- **High yield:** Elute with the volume indicated in the individual protocol and perform a second elution step with the first eluate as elution buffer.
- **Higher yield (maximum recovery):** Perform two elution steps with the respective buffer and volume indicated in the individual protocol (final concentration may be lower compared to the standard method).
- **High concentration:** Perform one elution step with 60 % of the volume indicated in the individual protocol (yield may be lower compared to the standard method).

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 15 – 25 °C and are stable until: see package label.
- Always keep buffer bottles tightly closed.
- Sodium dodecyl sulfate (SDS) in **Buffer A2** may precipitate if stored at temperatures below 20 °C. Precipitated SDS might form a firm layer at the bottom of the bottle which is difficult to see from the side or above. Invert the bottle carefully several times (avoid extensive foaming) and check the bottom and solution for white flocculates. If a precipitate is observed in Buffer A2, incubate bottle at 30 – 40 °C for several minutes and mix well.
- Buffer ERB may form crystals. The crystals have to be redissolved by heating to 50 – 60 °C whilst shaking. The bottle should be closed during the heat incubation at all times.
  Before starting the extraction process, the ERB buffer must be cooled down to room-temperature.

Before starting any **NucleoSpin® Plasmid Transfection-grade Plus** protocol, prepare the following:

- Add 3 mL of **Buffer A1** to the **RNase A** vial and mix by vortexing or pipetting up and down until the RNase A is resuspended completely. Transfer the solution back into the Buffer A1 bottle and mix thoroughly. Indicate date of RNase A addition and mark the corresponding checkbox of Buffer A1. 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**.

	NucleoSpin® Plasmid Transfection-grade Plus		
REF	**10 preps** **740500.10**	**50 preps** **740500.50**	**250 preps** **740500.250**
Wash Buffer AQ (Concentrate)	6 mL Add 24 mL ethanol	2 × 6 mL Add 24 mL ethanol to each bottle	2 × 25 mL Add 100 mL ethanol to each bottle

4 Safety instructions

When working with the **NucleoSpin® Plasmid Transfection-grade Plus** kits 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 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 Transfection-grade Plus** 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 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 Protocols

5.1 Isolation of transfection-grade plasmid DNA in single spin format

Before starting the preparation:

- Check if RNase A was added to Buffer A1 according to section 3.
- Check Lysis Buffer A2 for precipitated SDS according to section 3.
- Check Wash Buffer ERB for crystals according to section 3.
- Check if Wash Buffer AQ was prepared according to section 3.

1 Cultivate and harvest bacterial cells

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



11,000 × *g*, 30 s

2 Lyse cells

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!

Add **250 µL Buffer A2**. **Mix** gently by inverting the tube **6 – 8 times**. To avoid shearing of genomic DNA, do NOT vortex or pipette! Incubate at **room temperature** for up to **2 min** or until lysate appears clear.

Add **300 µL Buffer A3**. **Mix** thoroughly by **inverting the tube until the blue color disappeared completely** and an off-white precipitate has formed. To avoid shearing of genomic DNA, do NOT vortex or pipette!



+ 250 µL A1
Resuspend
+ 250 µL A2
Mix
RT, 2 min
+ 300 µL A3
Mix

3 Clarify lysate

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

Repeat this step in case the supernatant is not clear!



≥ 11,000 x *g*, 10 min

4 Bind DNA

Place a **NucleoSpin® Plasmid TG Plus Column** in a Collection Tube (2 mL) and decant or pipette the supernatant from step 3 onto the column. Make sure not to transfer cell debris as this results in a higher endotoxin concentration of the eluates.

Centrifuge for **1 min** at **11,000 × *g***. Discard flowthrough and place the **NucleoSpin® Plasmid TG Plus Column** back into the Collection Tube.



Load supernatant
11,000 × *g*, 1 min

5 Wash silica membrane

1st wash

Add **650 µL Buffer ERB**. Centrifuge for **1 min** at **11,000 × *g***. Discard flowthrough and place the NucleoSpin® Plasmid TG Plus Column back into the Collection Tube.



+ 650 µL ERB

11,000 × *g*, 1 min

2nd wash

Add **650 µL Buffer AQ**. Centrifuge for **1 min** at **11,000 × *g***. Discard flowthrough and place the NucleoSpin® Plasmid TG Plus Column back into the empty Collection Tube.



+ 650 µL AQ

11,000 × *g*, 1 min

6 Dry silica membrane

Centrifuge for **2 min** at **13,000 × *g*** and discard the Collection Tube.



13,000 × *g*, 1 – 2 min

7 Elute DNA

Place the NucleoSpin® Plasmid TG Plus Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL H_2O-EF**. Incubate for **1 min** at **room temperature**. Centrifuge for **2 min** at **13,000 × *g*.**



+ 50 µL H_2O-EF

RT, 1 min

13,000 × *g*, 2 min

<u>Note:</u> To ensure optimal plasmid yield or concentration for your subsequent application, refer to the elution procedures outlined in section 2.5.

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. Use the centrifugation speed and times given in the manual to avoid tight pellets. *SDS in Buffer A2 precipitated* • SDS in Buffer A2 may precipitate upon storage. If this happens a white precipitate is visible at the bottom of the bottle. Incubate Buffer A2 at 30 – 40 °C for several minutes and mix well before use. *Too many bacterial cells used* • Usage of LB as the growth medium is recommended. When using rich media like 2 x YT or TB, cultures may reach very high cell densities. Reduce culture volume.
Poor plasmid yield	*Incomplete lysis of bacterial cells* • See “Possible cause and suggestions” above *No plasmid contained in bacteria* • Cells carrying the plasmid of interest may become overgrown by non-transformed cells due to insufficient amounts of selective antibiotics. • Do not incubate cultures for more than 16 h as this may result in many dead and starving cells with degraded DNA. *Use of low-copy plasmid* • Getting acceptable plasmid yields for transfection requires high-copy plasmids in a miniprep scale or a switch to large scale kits (NucleoBond® Xtra Midi / Maxi). *Suboptimal elution conditions* • Elution efficiency will decrease with larger constructs. When working with large constructs, the elution buffer volume should be increased or the elution process repeated with the previous eluate as new elution buffer. • Silica and DNA bound thereto can be overdried by excess vacuum. Reduce vacuum force or time during the drying step and increase incubation times with H_2O-EF. *Buffer AQ not prepared correctly* • Add the indicated amount of 96 – 100 % ethanol to each bottle of Buffer AQ. Keep bottles closed tightly to prevent evaporation.

Problem	Possible cause and suggestions
Genomic DNA contamination	*Excessive mixing steps* • Cell lysate was vortexed or mixed too vigorously after addition of Buffer A2 or Buffer A3. Genomic DNA was sheared and thus liberated. *Lysis was too long* • Lysis was too long and must not exceed 5 min.
RNA contamination	*RNA was not degraded completely* • Ensure that RNase A was added to Buffer A1 and mixed well before use.
Suboptimal performance in downstream applications	*Carry-over of ethanol* • Make sure that the outlets do not come into contact with Buffer AQ after the drying step. *RNA contamination* • RNA might influence the photometric measurements resulting in an overestimation of plasmid DNA. Make sure RNase A is added to Buffer A1.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Plasmid Transfection-grade Plus	740500.10 740500.50 740500.250	10 preps 50 preps 250 preps
Buffer A1(without RNase A)	740911.1	1 L
Buffer A2 without Lyse Control	740912.1	1 L
Buffer A2 with LyseControl	740328.100	100 mL
Buffer A3	740913.1	1 L
Buffer AQ (Concentrate) (for 125 mL Buffer AQ)	740995	25 mL
Buffer ERB	740495.500 740495.1000	500 mL 1 L
H_2O-EF	740798.1	1 L
RNase A (lyophilized)	740505 740505.50	100 mg 50 mg
Collection Tubes (2 mL)	740600	1000

Visit ***www.mn-net.com*** for more detailed product information.

6.3 Product use restriction / warranty

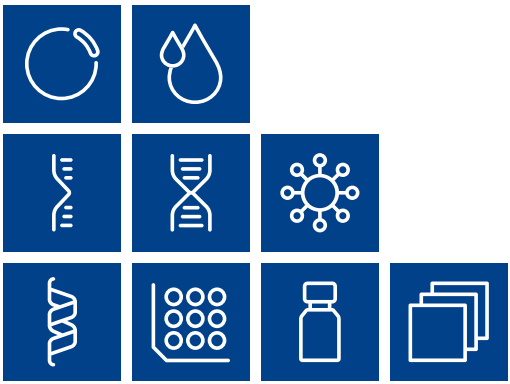
All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for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

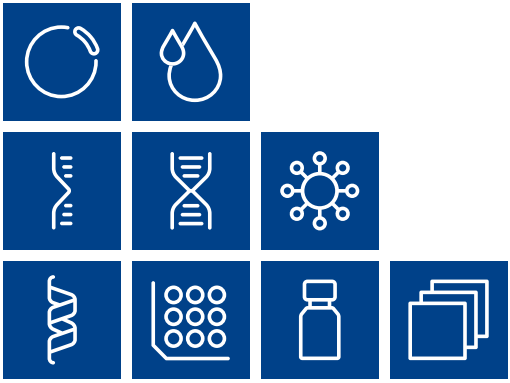
Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further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

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: + 49 24 21 969-333
support@mn-net.com



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



MACHEREY-NAGEL

www.mn-net.com

MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany

DE +49 24 21 969-0 info@mn-net.com
CH +41 62 388 55 00 sales-ch@mn-net.com
FR +33 388 68 22 68 sales-fr@mn-net.com
US +1 888 321 62 24 sales-us@mn-net.com