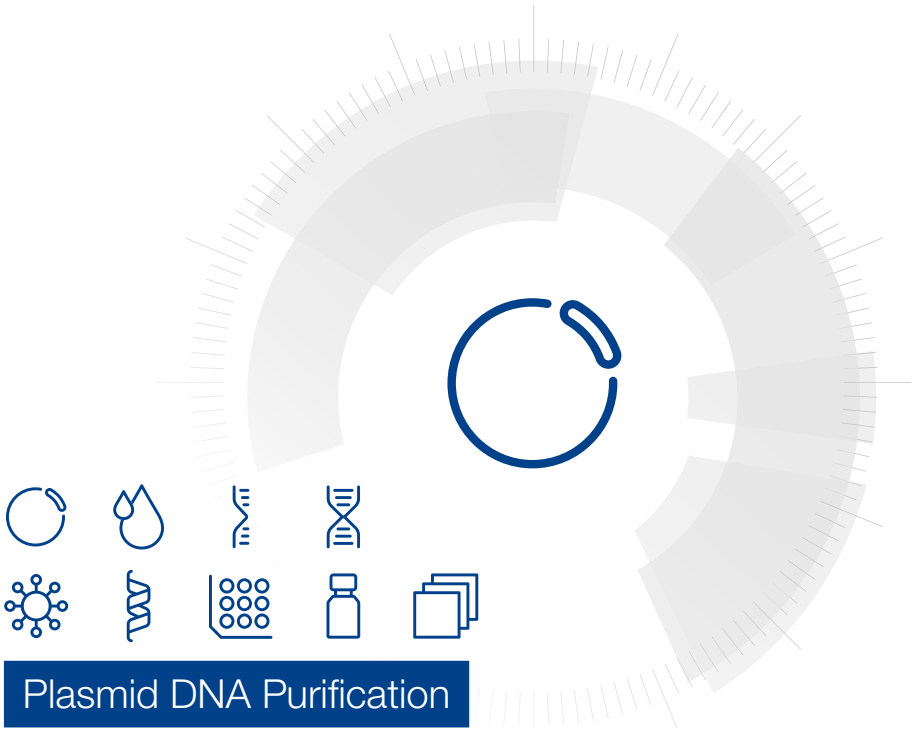
Bioanalysis

MACHEREY-NAGEL

# User manual



## Plasmid DNA Purification

■ NucleoSpin® Plasmid Transfection-grade Plus

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# 1 Components

## 1.1 Kit contents

| | NucleoSpin® Plasmid Transfection-grade Plus | | |
|---|---|---|---|
| REF | 10 preps<br>740500.10 | 50 preps<br>740500.50 | 250 preps<br>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 |
| Leafllet | 1 | 1 | 1 |

## 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

Equipment

- Centrifuge for microcentrifuge tubes

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

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

# 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 either 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*.

All technical literature is available on the internet at ***www.mn-net.com***.

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

**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 |
| Use | For research use only |

## 2.3 Required hardware

A suitable centrifuge for harvesting the bacteria (either plate or tube centrifuge) and for the elution step under centrifugation is required.

## 2.4 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.

## 2.5 Elution procedures

The single spin kit is optimized for an elution volume of 50 µL. A higher concentration without losses in recovery might be achieved by a second elution with the first eluate as elution buffer. To directly gain higher concentrations, the elution volume may be reduced to 30 µL.

In order to achieve maximum recovery, it is possible to perform a second elution using 50 µL of fresh $H_2O$-EF as the elution buffer. However, it should be noted that the final concentration achieved may be lower than in previous suggestions.

# 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 hypochloride). 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**<br>**740500.10** | **50 preps**<br>**740500.50** | **250 preps**<br>**740500.250** |
| Wash Buffer AQ (Concentrate) | 6 mL<br>Add 24 mL ethanol | 2 × 6 mL<br>Add 24 mL ethanol to each bottle | 2 × 25 mL<br>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**. Do not vortex or pipette to avoid shearing of genomic DNA.

Add **300 µL Buffer A3**. **Mix** thoroughly by **inverting the tube until the blue color disappeared completely** and an off-white precipitate has formed. Do not vortex to avoid shearing of genomic DNA.

**+ 250 µL A1**

**Resuspend**

**+ 250 µL A2**

**Mix**

**+ 300 µL A3**

**Mix**

**3 Clarify lysate**

Centrifuge for **10 min** at **full speed** at room temperature.

Repeat this step in case the supernatant is not clear!

**Full speed, 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 Columnback 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*.**

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

**+ 50 µL $H_2O$-EF**

**RT, 1 min**

**13,000 × *g*, 2 min**

# 6 Appendix

## 6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Incomplete lysis of bacterial cells | *Cell pellet not properly resuspended*<br>• 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.<br>*SDS in Buffer A2 precipitated*<br>• 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.<br>*Too many bacterial cells used*<br>• 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*<br>• See “Possible cause and suggestions” above<br>*No plasmid contained in bacteria*<br>• Cells carrying the plasmid of interest may become overgrown by non-transformed cells due to insufficient amounts of selective antibiotics.<br>• Do not incubate cultures for more than 16 h as this may result in many dead and starving cells with degraded DNA.<br>*Use of low-copy plasmid*<br>• 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).<br>*Suboptimal elution conditions*<br>• 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.<br>• 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.<br>*Buffer AQ not prepared correctly*<br>• 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*<br>• Cell lysate was vortexed or mixed too vigourously after addition of Buffer A2 or Buffer A3. Genomic DNA was sheared and thus liberated.<br>*Lysis was too long*<br>• Lysis was too long and must not exceed 5 min. |
| RNA contamination | *RNA was not degraded completely*<br>• Ensure that RNase A was added to Buffer A1 and mixed well before use. |
| Suboptimal performance in downstream applications | *Carry-over of ethanol*<br>• Make sure that the outlets do not come into contact with Buffer AQ after the drying step.<br>*RNA contamination*<br>• 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<br>740500.50<br>740500.250 | 10 preps<br>50 preps<br>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)<br>(for 125 mL Buffer AQ) | 740995 | 25 mL |
| $H_2O$-EF | 740798.1 | 1 L |
| RNase A (lyophilized) | 740505<br>740505.50 | 100 mg<br>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.

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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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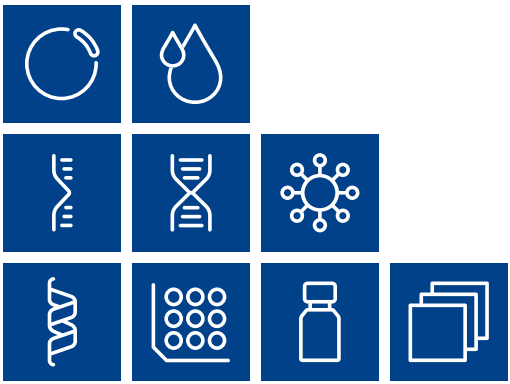
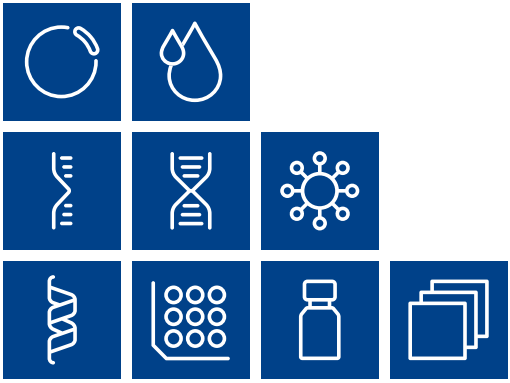


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools

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