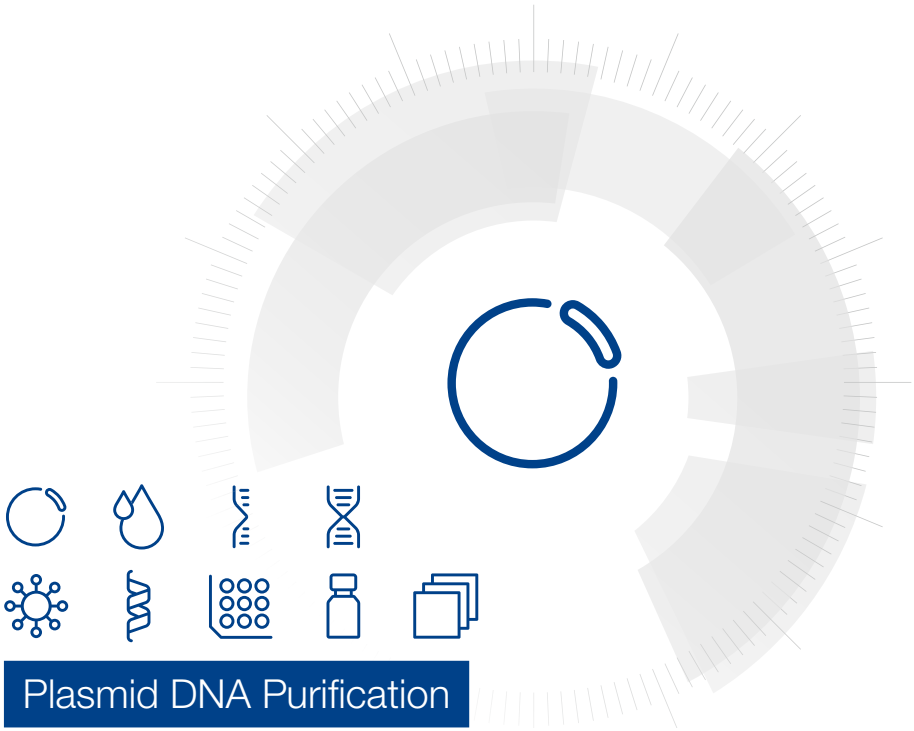
Bioanalysis

MACHEREY-NAGEL

User manual



Plasmid DNA Purification

- NucleoSpin® 96 Plasmid Transfection-grade Plus

September 2025 / Rev. 02

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1 Components

1.1 Kit contents

	NucleoSpin® 96 Plasmid Transfection-grade Plus	
REF	1 × 96 preps 740501.1	4 × 96 preps 740501.4
Resuspension Buffer A1	75 mL	150 mL
Lysis Buffer A2	100 mL	150 mL
Neutralization Buffer A3	100 mL	200 mL
Detoxification Buffer ERB	125 mL	400 mL
Wash Buffer AQ (Concentrate)*	100 mL	2 × 100 mL
H_2O-EF	30 mL	125 mL
RNase A (lyophilized)*	30 mg	60 mg
NucleoSpin® Plasmid Filter Plate (violet rings)	1	4
NucleoSpin® Plasmid TG Plus Binding Plate (orange rings)	1	4
Culture Plate (including Gas-permeable Foil)	1	4
MN Wash Plate	1	4
Rack of Tube Strips	1	4
Leaflet	1	1

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol

For more detailed information regarding special hardware required for centrifuge or vacuum processing, please see section 2.3.

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® 96 Plasmid Transfection-grade Plus** procedure is a modified version of the Birnboim and Doly[1] alkaline lysis plasmid miniprep protocol. Bacterial cultures are harvested by an initial centrifugation step. After resuspension of the pelleted bacteria (Buffer A1) and alkaline cell lysis (Buffer A2), a neutralization and binding buffer (Buffer A3) containing chaotropic salts is added. Resulting bacterial crude lysates are cleared by vacuum filtration with the NucleoSpin® Plasmid Filter Plate. The cleared lysates containing the plasmid DNA are collected into the NucleoSpin® Plasmid TG Plus Binding Plate without need of an additional transfer step allowing binding of plasmid DNA to NucleoSpin® Plasmid TG Plus Binding Plate during the second vacuum-filtration step.

High purity of the final plasmid DNA is achieved by removal of contaminants, especially endotoxins by the patented Detoxification Buffer ERB.

Further contaminations such as salts are removed with ethanolic Buffer AQ while traces of ethanol are removed by vacuum.

Highly pure plasmid DNA is eluted with endotoxin-free water and is ready for any common downstream application including transfection.

We can provide personalized support, protocol information, or verified scripts for numerous platforms. For more information, please contact our technical support or visit ***www.mn-net.com/automation***.

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

2.2 Kit specifications

The **NucleoSpin® 96 Plasmid Transfection-grade Plus** kits are designed for the rapid manual or automated small-scale purification of transfection-grade plasmid DNA in the 96-well plate format from *E. coli*.

The kits allow for easy automation on common liquid handling instruments. For more information about the automation process and the availability of ready-to-run scripts for certain platforms please refer to section 2.4 and / or contact your local distributor or MN directly.

- Using the NucleoSpin® 96 Plasmid kits allow simultaneous manual processing of up to 96 samples typically within less than 50 minutes. Actual processing time depends on the configuration of the liquid handling system used.
- Typically yields of 5 – 15 µg plasmid DNA can be purified from 1.5 mL overnight cultures.
- Yield depends on copy number and plasmid size, selected culture medium, and bacterial host strain.
- The maximum DNA binding capacity is 60 µg. The final concentration of the eluted DNA is typically within 50 – 600 ng/µL (depending on the elution buffer volume and the bacterial culture).
- Typically, the A_{260} / A_{280} ratio is > 1.8. Eluted DNA is ready to use for many downstream applications.

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

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® 96 Plasmid Transfection-grade Plus
Format	96-well plates
Processing	Manual or automated, vacuum or positive pressure
Sample material	≤ 5 mL *E. coli* culture
Vector size	< 25 kbp
Elution volume	75 – 150 µL
Preparation time	50 min/plate
Theoretical binding capacity	60 µg
Typical yield	15 – 50 µg
Typical endotoxin level	≤ 10 EU/µg with 3 wash steps
	≤ 1 EU/µg with 4 wash steps
Use	For research use only

2.3 Required hardware

This **NucleoSpin® 96 Plasmid Transfection-grade Plus** kits are intended for use under vacuum.

The **NucleoSpin® 96 Plasmid Transfection-grade Plus** kits can be used manually with the NucleoVac 96 Vacuum Manifold (see Ordering information, section 6.2). Additionally, a suitable centrifuge for harvesting the bacteria (either plate or tube centrifuge) is required.

2.4 Automated processing on robotic platforms

NucleoSpin® 96 Plasmid Transfection-grade Plus can be used fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adaptations to a certain workstation, please contact MN. Full processing under vacuum enables complete automation without the need of centrifugation steps, regarding the drying of the membrane and the elution step.

The risk of cross-contamination is reduced by optimized vacuum settings during the elution step and by the improved shape of the outlets of the NucleoSpin® Plasmid Binding Plate. Drying of the NucleoSpin® Plasmid Binding Plate under vacuum is sufficient because the bottom of the plate is protected from residues of wash buffer during the washing steps by the MN Wash Plate. As a result, we recommend trying to integrate the MN Wash Plate into the automated procedure. The MN Frame (see ordering information) can be used to position the disposable MN Wash Plate inside the vacuum chamber. This also reduces the risk of cross-contamination, as common metal adaptors tend to get contaminated by gDNA. Thorough cleaning of the vacuum chamber is recommended after each run to prevent forming of gDNA-containing aerosols.

The kit can be processed via positive pressure as well. We recommend to use either the MN positive pressure frame universal or the MN positive pressure frame MPE^2.

Visit MN online at ***www.mn-net.com*** or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup, instructions and selection of the protocol.

2.5 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** kits are 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, resulting in reduced yield.

2.5.1 Selection of culture media

The cultivation of cells is recommended at 37 °C in LB (Luria-Bertani) medium at constant shaking (200 – 250 rpm). Alternatively, rich media like 2 x YT or TB (Terrific Broth) can be used. By using 2 x YT or TB, bacteria grow faster and reach the stationary phase much earlier than in LB medium (≤ 12 h) in culture tubes or flasks. This may lead to a higher percentage of dead or starving cells when starting the preparation. The resulting plasmid DNA from overgrown cultures may be partially degraded or contaminated with chromosomal DNA.

2.5.2 Cultivation of bacteria in a Square-well Block

Use the 96-well Square-well Block / Culture Plate for growing bacteria. Add 1.2 – 1.5 mL of selected medium (with appropriate antibiotic, e.g., 100 µg/mL ampicillin) to each well of the Square-well Block. To avoid cross-contamination due to spillage during incubation, do not exceed a total culture volume of 1.5 mL. Inoculate each

well with a single bacterial colony. Cover the Square-well Block with the Gas-permeable Foil. Grow the culture in a suitable incubator at 37 °C for 16 – 24 h with vigorous shaking (200 – 400 rpm). The Square-well Block may be fixed to the shaker with large-size flask clamps (for 2-L flasks) or tape.

Note: The yield of plasmid DNA depends on growth conditions, bacterial strain, and cell density of the culture as well as on the size and copy number of the vector. Use of highcopy number plasmids such as pUC, pBluescript, or pGEM and E. coli strains like DH5α, TOP10 or XL1 Blue are recommended. Growth times of 16 – 24 h are usually sufficient. However, for poorly growing bacteria, prolonged incubation times of up to 30 h may be required.

If an increased total culture volume is desired (< 1.5 mL), it is possible to grow bacteria in several Culture Plates with identical layout or in 24 well plates. Either way take care not to exceed the total resuspension volume of 250 µL per sample. The total volume of 250 µL per sample may either be split into the corresponding amount of plates and pooled after resuspension or the total amount may directly be dispensed into a first plate and completely transferred to succeeding plates after resuspension.

2.5.3 Cultivation of bacteria in tubes

Use 1 – 5 mL of appropriate culture medium. Depending on the bacterial strain and copy number of the plasmid, up to 5 mL LB medium or 2.5 mL 2 x YT or 2.5 mL TB medium can be used. Grow bacteria with vigorous shaking (200 – 250 rpm) for 10 – 14 h.

Optional: If the liquid handling instrument does not allow for the use of selected culture tubes, transfer the bacterial culture from the tubes into a suitable Square-well Block. For this, transfer 1.5 mL of the culture to each well of the Square-well Block. Harvest the

cultures by centrifugation. Discard supernatant. Usually 1.5 mL of culture are sufficient for DNA preparation. However, if necessary, add additional 1.0 – 1.5 mL bacterial culture to each well of the Square-well Block, centrifuge again, and discard the supernatant.

Do not use more than 5 mL LB culture or 2.5 mL rapid growing bacterial strain (using 2 x YT or TB medium)

2.6 Elution procedures

Elution efficiency depends on vector size and elution volume.

Elution under vacuum is more prone to losses in elution volume due to the technical dead volume. See the following table for correlation between the dispensed elution buffer volume and typical recoveries following the standard protocol under vacuum.

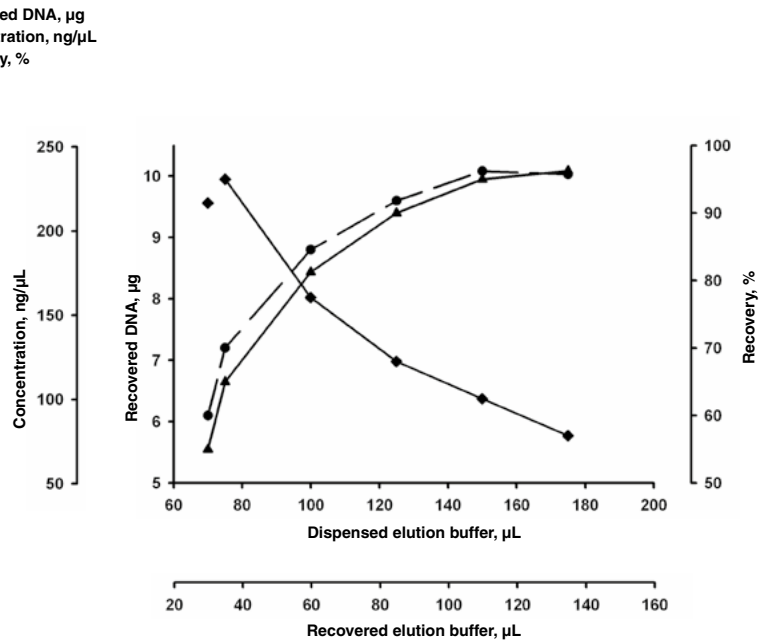


Figure 1: Recovery rate and concentration depend on elution volume.

10 µg of pBluescript plasmid were purified with NucleoSpin® 96 Plasmid and eluted with the indicated elution buffer volumes.

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® 96 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® 96 Plasmid Transfection-grade Plus	
REF	**1 × 96 prep** **740501.1**	**4 × 96 preps** **740501.4**
Wash Buffer AQ (Concentrate)	100 mL Add 400 mL ethanol	2 × 100 mL Add 400 mL ethanol to each bottle

4 Safety instructions

When working with the **NucleoSpin® 96 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® 96 Plasmid Transfection-grade Plus** kits 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 Protocol

5.1 NucleoSpin® 96 Plasmid Transfection-grade Plus Manual vacuum processing

- For hardware requirements, refer to section 2.3.
- Fore detailed information regarding the vacuum manifold setup, see page 14 – 23

For processing of the **NucleoSpin® 96 Plasmid Transfection-grade Plus** under vacuum the NucleoVac 96 Vacuum Manifold is required (see Ordering information, section 6.2).

Before starting the preparation:

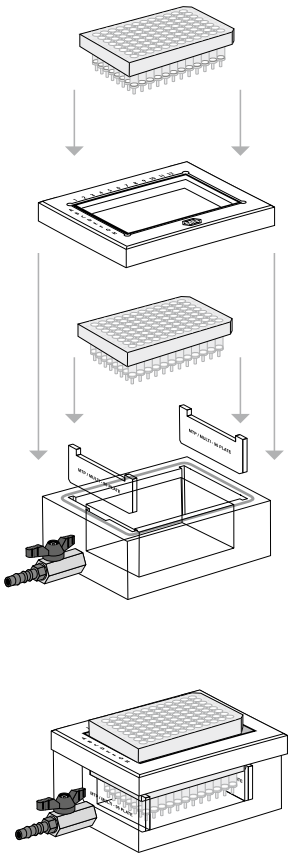
- Check if RNase A was added to Buffer A1 according to section 3.
- Check Lysis Buffer A2 for precipitated SDS according to section 3.
- Check Wash Buffer ERB for crystals according to section 3.
- Check if Wash Buffer AQ was prepared according to section 3.
- Set up the vacuum according to the scheme.

Protocol at a glance

1	**Cultivate** and **harvest** bacterial cells	**1.5 – 5 mL LB or up to 2.5 mL 2x YT or TB** **10 min, 1,000 x *g***
2	**Resuspend** bacterial cells	**250 µL A1** **Mix or shake**
3	**Lyse** bacterial cells	**250 µL A2** **RT, 2 – 5 min** **Shake**
4	**Neutralize**	**350 µL A3** **Mix or shake** **Prepare vacuum manifold for lysate clearing step**
5	**Transfer** crude lysate to NucleoSpin® Plasmid Filter Plate (violet rings)	
6	**Clear** crude lysate by vacuum filtration directly into the NucleoSpin® Plasmid TG Plus Binding Plate (orange rings) Optional: Incubate 1 – 3 min before applying vacuum	**-0.2 – 0.4 bar*, 1 – 5 min**
7	**Reassemble** vacuum manifold Discard NucleoSpin® Plasmid Filter Plate Remove NucleoSpin® Plasmid TG Plus Binding Plate with cleared lysates and insert MN Wash Plate Place the NucleoSpin® Plasmid TG Plus Binding Plate on top of the manifold	
8	**Bind** DNA to silica membrane of the NucleoSpin® Plasmid TG Plus Binding Plate by applying vacuum	**-0.2 – 0.4 bar*, 1 min**
9	**Wash** silica membrane	**900 µL ERB** **900 µL AQ** **900 µL AQ** **-0.2 to -0.4 bar*, 1 – 2 min each step**
10	**Remove** MN Wash Plate	
11	**Dry** NucleoSpin® Plasmid TG Plus Binding Plate by applying vacuum *Optional: Dry the outlets of the NucleoSpin® Plasmid TG Plus Binding Plate by placing it on a sheet of filter paper before applying vacuum*	**Full vacuum, 10 – 15 min**
12	**Insert** Rack of Tube Strips	
13	**Elute** plasmid DNA	**2 × 75 µL H_2O-EF or 1 × 150 µL H_2O-EF** **Incubate, RT, 3 min** **-0.4 to 0.6 bar, 1 min**

Setup of vacuum manifold: Lysate clearing

Lysate clearing



Step 4:
Place the NucleoSpin® Filter Plate on top of the manifold.

Step 3:
Place the manifold lid on top of the manifold base.

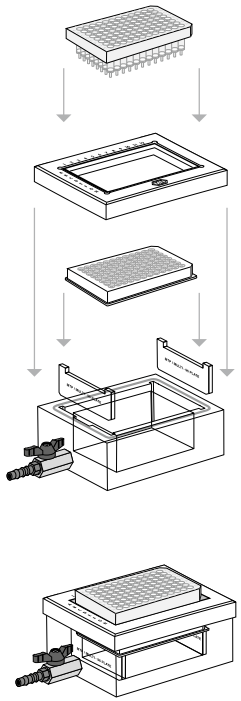
Step 2:
Place the NucleoSpin® Plasmid TG Plus Binding Plate into the manifold.

Step 1:
Insert spacers`MTP/Multi-96 Plate‘ in the manifold base.

Final setup

Setup of vacuum manifold: Binding / Washing / Elution steps

Binding / Washing steps



Step 4:
Place the NucleoSpin® Plasmid TG Plus Binding Plate on top of the manifold lid.

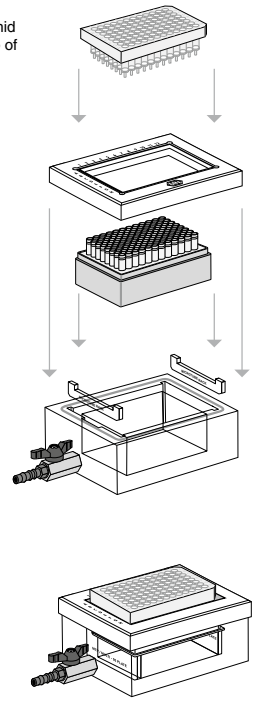
Step 3:
Place the manifold lid on top of the manifold base.

Step 2:
Place the MN Wash Plate in the manifold.

Step 1:
Insert spacers ‘MTP/MULTI-96 PLATE‘ in the manifold base.

Final setup

Elution step



Step 4:
Place the NucleoSpin® Plasmid TG Plus Plate on top of the manifold lid.

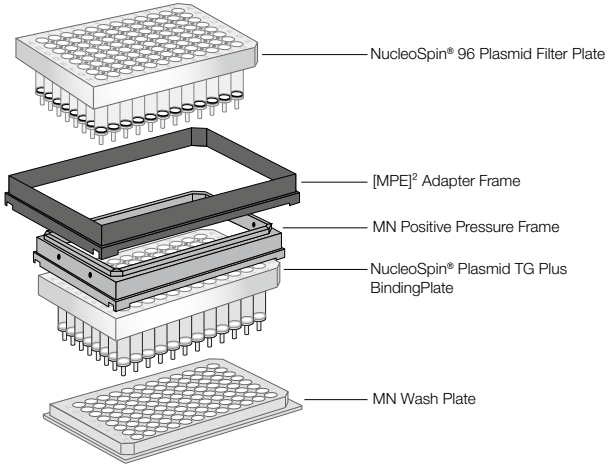
Step 3:
Place the manifold lid on top of the manifold base.

Step 2:
Place the Rack of Tube Strips in the manifold.

Step 1:
Insert spacers ‘Microtube Rack‘ in the manifold base.

Final setup

Setup of MN Positive Pressure Frame and [MPE]² Adapter Frame: Binding / Washing / Elution steps



Detailed protocol

For processing of the **NucleoSpin® 96 Plasmid Transfection-grade Plus** under vacuum the NucleoVac 96 Vacuum Manifold is required (see Ordering information, section 6.2).

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.
- Set up the vacuum according to the scheme.

1 Cultivate and harvest bacterial cells

Centrifuge the bacterial cultures for **10 min** at **1,000 x *g***.

Centrifugation at higher *g*-forces might produce tight pellets which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

2 Resuspend bacterial cells

Add **250 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing or mixing by pipetting up and down. Resuspend bacterial cells completely before addition of Buffer A2!

3 Lyse bacterial cells

Add **250 µL Buffer A2**. Do not vortex or pipette to avoid shearing of genomic DNA.

Incubate at **room temperature** for a maximum of **5 min** with moderate shaking (300 rpm).

Note: Do not vortex; doing so will release contaminating chromosomal DNA from the cellular debris into the suspension. Do not allow the lysis reaction to proceed for more than 5 minutes.

4 Neutralize

Add **350 µL Buffer A3**. Mix by pipetting up and down. Do not vortex to avoid shearing of genomic DNA.

5 Prepare the NucleoVac 96 Vacuum Manifold

Prepare the manifold for filtration of crude lysates:

Insert spacers labeled 'MTP/Multi-96 Plate' notched side up into the grooves located on the short side of the manifold base. Insert waste container into manifold base. Place the NucleoSpin® Plasmid TG Plus Binding Plate (orange rings) on top of the spacers. Insert the NucleoSpin® Plasmid Filter plate (violet rings) into the manifold lid and place the lid on the manifold base. Close the manifold base with the manifold lid.

6 Transfer crude lysates onto the NucleoSpin® Plasmid Filter Plate

Transfer crude lysates from step 4 carefully and completely into the wells of the NucleoSpin® Plasmid Filter Plate.

Note: Mix the suspension by pipetting up and down the entire volume once before transfer to the NucleoSpin® Plasmid Filter Plate.

7 Clear crude lysate by vacuum filtration

Apply vacuum of **-0.2 to -0.4 bar* (1 – 5 min)**. If necessary, press down the NucleoSpin® Plasmid Filter Plate slightly until flowthrough starts. Adjust vacuum to establish a flow rate of 1 – 2 drops per second.

When the crude lysate has passed the NucleoSpin® Plasmid Filter Plate, release the vacuum.

8 Reassemble vacuum manifold

Remove and discard the NucleoSpin® Plasmid Filter Plate. Open the manifold lid. Remove the NucleoSpin® Plasmid TG Plus Binding Plate (orange rings) with cleared lysates.

Insert waste container into manifold base. Insert the MN Wash Plate onto the spacers inside the manifold base. Close the manifold base with the manifold lid. Place the NucleoSpin® Plasmid TG Plus Binding Plate on top of the manifold.

9 Bind DNA to silica membrane

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

When the cleared lysate has passed the NucleoSpin® Plasmid TG Plus Binding Plate, release the vacuum.

* Reduction of atmospheric pressure

10 Wash silica membrane

1st wash

Add **900 µL Buffer ERB** to each well. Apply vacuum of **-0.2 to -0.4 bar* (2 min)**.

Release the vacuum once the buffer has passed all wells.

2nd wash

Add **900 µL Buffer AQ** to each well. Apply vacuum of **-0.2 to -0.4 bar* (1 min)**.

Release the vacuum once the buffer has passed all wells.

3rd wash

Repeat the wash step with **900 µL Buffer AQ**. Apply vacuum of **-0.2 to -0.4 bar* (1 min)**.

Release the vacuum once the buffer has passed all wells.

11 Remove MN Wash Plate

After the final washing step, remove the NucleoSpin® Plasmid TG Plus Binding Plate. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

12 Dry silica membrane

Remove any residual wash buffer from the NucleoSpin® Plasmid TG Plus Binding Plate. If necessary, tap the outlets of the plate onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue.

Close the manifold base with the manifold lid. Place the NucleoSpin® Plasmid TG Plus Binding Plate on top of the manifold.

Apply vacuum of **-0.4 to -0.6 bar*** for **10 – 15 min** to dry the membrane completely. Run vacuum pump continuously. Typically, the adjusted vacuum is not reached at this step. Achieving and keeping a continuous air-flow in order to evaporate the remaining ethanol from Wash Buffer AQ is of more importance than reaching the precise mentioned atmospheric pressure.

Note: The ethanol in Buffer AQ inhibits enzymatic reactions and has to be completely removed before eluting the DNA.

Finally, release the vacuum.

13 Insert Rack of Tubes Strips

Remove the manifold lid with the NucleoSpin® Plasmid TG Plus Binding Plate from the vacuum manifold. Insert the Rack of Tubes Strips on the spacers inside the manifold base. Close the manifold base with the manifold lid. Place the NucleoSpin® Plasmid TG Plus Binding Plate (orange rings) on top of the manifold.

Note: To elute into the Rack of Tube Strips, 'Microtube Rack' spacers are required. These must be replaced with the spacers used in the manifold base of the previous steps.

14 Elute DNA

Elute the DNA by adding **2 × 75 µL or 1 × 150 µL H_2O-EF** (150 µL is recommended, a volume range of 75 – 150 µL is possible, see section 2.6) or sterile distilled water (pH 7.5 – 8.5) to each well of the NucleoSpin® Plasmid TG Plus Binding Plate.

The elution buffer should be dispensed carefully onto the center of the silica membrane. Incubate the buffer on the membrane for **3 min** at room temperature. Apply vacuum of **-0.4 to -0.6 bar* (5 min)**. If necessary, press down the NucleoSpin®R Plasmid TG Plus Binding Plate slightly and collect the eluted DNA. Take care not to generate an airflow that is too forceful, as spilling of eluates might lead to cross-contamination and loss of DNA.

After the elution buffer has passed the wells, release vacuum. Remove the Rack of Tube Strips containing eluted DNA and seal the strips with Cap Strips for further storage.

5.2 Support protocol for endotoxin free plasmid DNA isolation

The following support protocol was designed to achieve plasmid DNA purifications with a typical endotoxin level ≤ 1 EU / µg Plasmid DNA. However, please note that a reduction in product yield of 5 – 10 % compared to the standard protocol may occur.

If endotoxin levels of ≤ 10 EU/µg are sufficient, it is therefore recommended to use

the standard procedure.

The amount of Wash Buffer ERB included in the kit is only sufficient for the standard protocol. **In order to carry out this procedure, an additional amount of buffer ERB is necessary, which can be purchased separately. Please refer to chapter 6.2 for ordering information.**

The protocol remains consistent with the standard protocol described in section (see NucleoSpin® 96 Plasmid Transfection-grade Plus Manual vacuum processing, section 5.1), with the exception that it includes an additional washing step. In step 10, an additional ERB wash step must be performed, resulting in a total of four washing steps

1

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.
- Check whether additional Wash Buffer ERB has been purchased for the preparation of the support protocol. For ordering information please refer to chapter (see Ordering information, section 6.2).
- Set up the vacuum according to the scheme.

2 Cultivate and harvest bacterial cells

Centrifuge the bacterial cultures for **10 min** at **1,000 x *g***.

Centrifugation at higher *g*-forces might produce tight pellets which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

3 Resuspend bacterial cells

Add **250 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing or mixing by pipetting up and down. Resuspend bacterial cells completely before addition of Buffer A2!

4 Lyse bacterial cells

Add **250 µL Buffer A2**. Do not vortex or pipette to avoid shearing of genomic DNA.

Incubate at **room temperature** for a maximum of **5 min** with moderate shaking (300 rpm).

Note: Do not vortex; doing so will release contaminating chromosomal DNA from the cellular debris into the suspension. Do not allow the lysis reaction to proceed for more than 5 minutes.

5 Neutralize

Add **350 µL Buffer A3**. Mix by pipetting up and down. Do not vortex to avoid shearing of genomic DNA.

6 Prepare the NucleoVac 96 Vacuum Manifold

Prepare the manifold for filtration of crude lysates:

Insert spacers labeled 'MTP/Multi-96 Plate' notched side up into the grooves located on the short side of the manifold base. Insert waste container into manifold base. Place the NucleoSpin® Plasmid TG Plus Binding Plate (orange rings) on top of the spacers. Insert the NucleoSpin® Plasmid Filter plate (violet rings) into the manifold lid and place the lid on the manifold base. Close the manifold base with the manifold lid.

7 Transfer crude lysates onto the NucleoSpin® Plasmid Filter Plate

Transfer crude lysates from step 4 carefully and completely into the wells of the NucleoSpin® Plasmid Filter Plate.

Note: Mix the suspension by pipetting up and down the entire volume once before transfer to the NucleoSpin® Plasmid Filter Plate.

8 Clear crude lysate by vacuum filtration

Apply vacuum of **-0.2 to -0.4 bar* (1 – 5 min)**. If necessary, press down the NucleoSpin® Plasmid Filter Plate slightly until flowthrough starts. Adjust vacuum to establish a flow rate of 1 – 2 drops per second.

When the crude lysate has passed the NucleoSpin® Plasmid Filter Plate, release the vacuum.

9 Reassemble vacuum manifold

Remove and discard the NucleoSpin® Plasmid Filter Plate. Open the manifold lid. Remove the NucleoSpin® Plasmid TG Plus Binding Plate (orange rings) with cleared lysates.

Insert waste container into manifold base. Insert the MN Wash Plate onto the spacers inside the manifold base. Close the manifold base with the manifold lid. Place the NucleoSpin® Plasmid TG Plus Binding Plate on top of the manifold.

* Reduction of atmospheric pressure

10 Bind DNA to silica membrane

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

When the cleared lysate has passed the NucleoSpin® Plasmid TG Plus Binding Plate, release the vacuum.

11 Wash silica membrane

1st wash

Add **900 µL Buffer ERB** to each well. Apply vacuum of **-0.2 to -0.4 bar* (2 min)**. Release the vacuum once the buffer has passed all wells.

2nd wash

Repeat the wash step with **900 µL Buffer ERB**. Apply vacuum of **-0.2 to -0.4 bar* (2 min)**. Release the vacuum once the buffer has passed all wells.

3rd wash

Add **900 µL Buffer AQ** to each well. Apply vacuum of **-0.2 to -0.4 bar* (1 min)**. Release the vacuum once the buffer has passed all wells..

4th wash

Repeat the wash step with **900 µL Buffer AQ**. Apply vacuum of -**0.2 to -0.4 bar* (1 min)**.

Release the vacuum once the buffer has passed all wells.

12 Remove MN Wash Plate

After the final washing step, remove the NucleoSpin® Plasmid TG Plus Binding Plate. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

13 Dry silica membrane

Remove any residual wash buffer from the NucleoSpin® Plasmid TG Plus Binding Plate. If necessary, tap the outlets of the plate onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue.

Close the manifold base with the manifold lid. Place the NucleoSpin® Plasmid TG Plus Binding Plate on top of the manifold.

Apply vacuum of **-0.4 to -0.6 bar*** for **10 – 15 min** to dry the membrane completely. Run vacuum pump continuously. Typically, the adjusted vacuum is not reached at this step. Achieving and keeping a continuous air-flow in order to evaporate the remaining ethanol from Wash Buffer AQ is of more importance than reaching the precise mentioned atmospheric pressure.

Note: The ethanol in Buffer AQ inhibits enzymatic reactions and has to be completely removed before eluting the DNA.

Finally, release the vacuum.

14 Insert Rack of Tubes Strips

Remove the manifold lid with the NucleoSpin® Plasmid TG Plus Binding Plate from the vacuum manifold. Insert the Rack of Tubes Strips on the spacers inside the manifold base. Close the manifold base with the manifold lid. Place the NucleoSpin® Plasmid TG Plus Binding Plate (orange rings) on top of the manifold.

Note: To elute into the Rack of Tube Strips, 'Microtube Rack' spacers are required. These must be replaced with the spacers used in the manifold base of the previous steps.

15 Elute DNA

Elute the DNA by adding **2 × 75 µL or 1 × 150 µL H_2O-EF** (150 µL is recommended, a volume range of 75 – 150 µL is possible, see section 2.6) or sterile distilled water (pH 7.5 – 8.5) to each well of the NucleoSpin® Plasmid TG Plus Binding Plate.

The elution buffer should be dispensed carefully onto the center of the silica membrane. Incubate the buffer on the membrane for **3 min** at room temperature. Apply vacuum of **-0.4 to -0.6 bar* (5 min)**. If necessary, press down the NucleoSpin®R Plasmid TG Plus Binding Plate slightly and collect the eluted DNA. Take care not to generate an airflow that is too forceful, as spilling of eluates might lead to cross-contamination and loss of DNA.

After the elution buffer has passed the wells, release vacuum. Remove the Rack of Tube Strips containing eluted DNA and seal the strips with Cap Strips for further storage.

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.

Problem	Possible cause and suggestions
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 of H_2O-EF. *Eluate spillage* • Increase the vacuum force carefully when eluting the DNA into Elution Plates U-bottom. Watch the eluates while increasing the vacuum force. *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 vigourously after addition of Buffer A2 or Buffer A3. Genomic DNA was sheared and thus liberated. • Reduce number of mixing cycles, reduce shaker speed after addition of Lysis Buffer A2 and Neutralization Buffer A3 or before transfer of crude lysates to the NucleoSpin® Plasmid Filter Plate. Mixing will cause shearing of chromosomal DNA, leading to a copurification during the preparation of plasmid DNA. Use wide bore disposable tips for transfer of crude lysate to the NucleoSpin® Plasmid Filter plate to prevent shearing of chromosomal DNA. *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® 96 Plasmid Transfection-grade Plus	740501.1 740501.4	1 × 96 preps 4 × 96 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	740495.1000 740495.500	1 L 500 mL
H_2O-EF	740798.1	1 L
RNase A (lyophilized)	740505 740505.50	100 mg 50 mg
NucleoVac 96 Vacuum Manifold	740681	1 piece
NucleoVac Vacuum Regulator	740641	1 piece
Rack of Tube Strips (1 set consists of 1 rack, 12 strips with 8 tubes each and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Cap Strips	740478 740478.24	48 pieces 288 pieces
MN Square-well Block	740476 740476.24	4 pieces 24 pieces
MN Wash Plate	740479 740479.24	4 pieces 24 pieces
Culture Plate (with Gas-permeable Foil)	740488 740488.24	4 sets 24 sets
Elution Plate U-bottom (with Self adhering Foil)	740486.24	24 sets
Gas-permeable Foil	740675	50 pieces
Self adhering Foil	740676	50 pieces
MN Frame	740680	1 piece
MN Positive Pressure Frame MPE^2	740474	1 piece
MN Positive Pressure Frame Universal	740497	1 piece

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.

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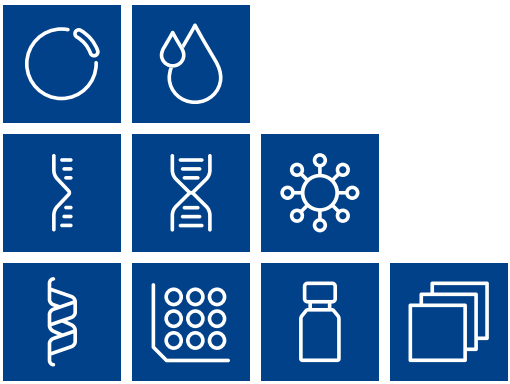
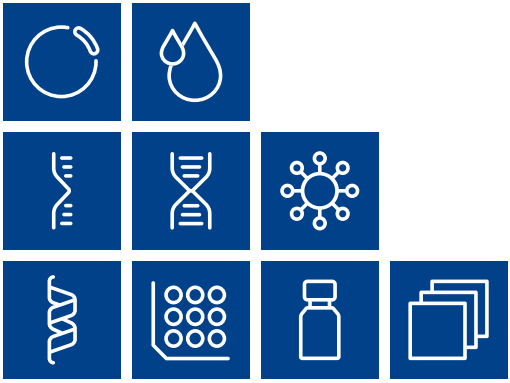


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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