

Plasmid DNA purification

User manual

NucleoSpin[®] 8 Plasmid

NucleoSpin[®] 8 Plasmid Core Kit

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MACHEREY-NAGEL

www.mn-net.com



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1 Components

1.1 Kit contents

| REF | NucleoSpin® 8 Plasmid | |
|---|------------------------|--------------------------|
| | 12 x 8 preps 740621 | 60 x 8 preps 740621.5 |
| Resuspension Buffer A1 | 75 mL | 150 mL |
| Lysis Buffer A2 | 75 mL | 150 mL |
| Neutralization Buffer A3 | 100 mL | 200 mL |
| Wash Buffer AW | 100 mL | 500 mL |
| Wash Buffer A4 (Concentrate) ¹ | 100 mL | 4 x 100 mL |
| Elution Buffer AE ² | 30 mL | 125 mL |
| RNase A (lyophilized) ¹ | 30 mg | 60 mg |
| NucleoSpin® Plasmid Binding Strips (transparent rings) | 12 | 60 |
| NucleoSpin® Plasmid Filter Strips (purple rings) | 12 | 60 |
| Culture Plate (including Gas- permeable Foil) | 1 | 5 |
| Rack of Tube Strips ³ | 1 | 5 |
| MN Wash Plate | 1 | 5 |
| User manual | 1 | 1 |

¹ For preparation of working solutions and storage conditions see section 3.

² Composition of Elution Buffer AE: 5 mM Tris/HCl, pH 8.5

³ 1 rack = 12 strips with 8 tubes each, Cap Strips included

| NucleoSpin® 8 Plasmid Core Kit | |
|---|----------------------------------|
| REF | 48 x 8 preps 740461.4 |
| Resuspension Buffer A1 | 150 mL |
| Lysis Buffer A2 | 150 mL |
| Neutralization Buffer A3 | 200 mL |
| Wash Buffer A4 Concentrate) ¹ | 2 x 100 mL |
| Elution Buffer AE ² | 125 mL |
| RNase A (lyophilized) ¹ | 60 mg |
| NucleoSpin® Plasmid Binding Strips (transparent rings) | 48 |
| NucleoSpin® Plasmid Filter Strips (purple rings) | 48 |
| User manual | 1 |

1.2 Reagents to be supplied by user

- 96–100 % ethanol

¹ For preparation of working solutions and storage conditions see section 3.

² Composition of Elution Buffer AE: 5 mM Tris/HCl, pH 8.5

2 Product description

2.1 The basic principle

The **NucleoSpin® 8 Plasmid** procedure is a modified version of the Birnboim and Doly¹ alkaline lysis plasmid Mini prep 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 Strips. The cleared lysates containing the plasmid DNA are collected into the NucleoSpin® Plasmid Binding Strips. The chaotropic salt leads to a reversible adsorption of the plasmid DNA to the NucleoSpin® silica membrane during the second vacuum-filtration step. High purity of the final plasmid DNA preparation is achieved by complete removal of cellular contaminants, salts, detergents, and other compounds by subsequent washing steps. Highly pure plasmid DNA is finally eluted with Elution Buffer AE (5 mM Tris/HCl, pH 8.5) or water (pH 8.0–8.5) and can directly be used for downstream applications.

2.2 Kit specifications

- **NucleoSpin® 8 Plasmid** is designed for the manual or automated small-scale purification of high-copy plasmid DNA from *E. coli* in the flexible 8-well strip format.
- **NucleoSpin® 8 Plasmid** kits (REF 740621 /.5) are supplied with all accessory plates for highest convenience.
- The **NucleoSpin® 8 Plasmid Core Kit** (REF 740461.4) provides the buffers, RNase A, NucleoSpin® Plasmid Filter Strips, and NucleoSpin® Plasmid Binding Strips. Accessory components (e.g., culture plates, elution plates, MN Wash Plate, and Wash Buffer AW) are not provided with the core kit but can be individually selected from a variety of suitable accessories (see section 2.4 for further information). This allows highest flexibility for the user.
Please note: All given specifications or information in this manual refer equally to the **NucleoSpin® 8 Plasmid** kit (REF 740621 /.5) as well as to the **NucleoSpin® 8 Plasmid Core Kit** (REF 740461.4).
- 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 2.5 and / or contact your local distributor or MN directly.
- Using the **NucleoSpin® 8 Plasmid** kits allows simultaneous manual processing of up to 48 samples typically within less than 45 minutes. Actual processing time depends on the configuration of the liquid handling system used.

¹ Birnboim, H.C. & Doly, J. (1979) *Nucleic Acids Res.* 7, 1513-1523.

- 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 DNA binding capacity is about 20 µg. The final concentration of the eluted DNA is 50–200 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.

| Kit specifications at a glance | |
|---------------------------------------|----------------------------------|
| Parameter | NucleoSpin® 8 Plasmid |
| Format | 8-well strips |
| Processing | Manual or automated, vacuum |
| Lysate clarification | 8-well filter strips |
| Sample material | 1–5 mL <i>E. coli</i> culture |
| Vector size | <15 kbp |
| Typical yield | 4–6 µg/mL <i>E. coli</i> culture |
| Elution volume | 75–150 µL |
| Preparation time | 45 min/6 strips |
| Binding capacity | 20 µg |

2.3 Required hardware

This kit is intended for use under vacuum. A support protocol for elution under centrifugation is included (see section 5.2).

A support protocol for complete processing under centrifugation is available from our technical service (tech-bio@mn-net.com).

Vacuum processing

The **NucleoSpin® 8 Plasmid** kits can be used **manually** with the NucleoVac 96 Vacuum Manifold (see ordering information). Additionally, a suitable centrifuge for harvesting the bacteria (either plate or tube centrifuge) and for the optional elution step under centrifugation is required.

For processing the 8-well strips under vacuum, the **Starter Set A** (see ordering information) containing Column Holders A and NucleoSpin® Dummy Strips is required.

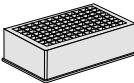
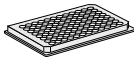

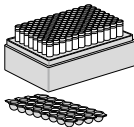
For **automation** on laboratory platforms with standard 96-well plate manifolds, Starter Set A is also required

2.4 Recommended accessories for use of the NucleoSpin® 8 Plasmid Core Kit

The **NucleoSpin® 8 Plasmid Core Kit** provides all buffers (except optional Wash Buffer AW), RNase A, and NucleoSpin® Filter/Binding Strips. Accessory plates (e.g., culture blocks, elution plates) are not provided with the core kit. The condensed kit composition, along with a large variety of separately available accessories, allows for an optimal adjustment of the kit to individual user needs. The user can select additional consumables according to his / her requirements for highest flexibility.

For use of **NucleoSpin® 8 Plasmid Core Kit**, follow the standard protocols (see section 5.1 or 5.2, respectively).

Recommended accessories for use of the **NucleoSpin® 8 Plasmid Core Kit** are available from MACHEREY-NAGEL. For ordering information, please refer to section 6.2.

| Protocol step | Suitable consumables, not supplied with the core kits | Remarks |
|-------------------------|--|---|
| Cultivation of bacteria | Culture Plates  | Square-well Blocks with Gas-permeable Foil |
| Wash step | MN Wash Plates  Buffer AW | MN Wash Plate minimizes the risk of cross contamination (vacuum processing only) Recommended additional wash buffer for bacterial host strain with high endogenous nuclease activity (e.g., <i>E. coli</i> HB 101, BMH 71-18 mutS, JM, or any wildtype strains) or for improvement of sequencing results |
| Elution | Elution Plate U-bottom  or Rack of Tube Strips (including Cap Strips)  | Not suitable for elution by centrifugation |

2.5 Automated processing on robotic platforms

NucleoSpin® 8 Plasmid can be used fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 8 Plasmid** on a certain workstation, please contact MN. Full processing under vacuum enables complete automation without the need of centrifugation steps, regarding the drying of the binding membrane and 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 Strips.

Drying of the NucleoSpin® Plasmid Binding Strips under vacuum is sufficient because the bottom of the strip is protected from buffer residues 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 gDNA-containing aerosols from forming.

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. Several application notes of the **NucleoSpin® 8 Plasmid** kit on various liquid handling instruments can also be found at www.mn-net.com under Bioanalysis/Literature.

2.6 Growth of bacterial cultures

2.6.1 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 2x YT or TB (Terrific Broth) can be used. By using 2x 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.

2.6.2 Cultivation of bacteria in a Square-well Block

Use the 96-well Square-well Block (Culture Plate; not included in the core kits) 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 high-copy number plasmids such as pUC, pBluescript, or pGEM and E. coli strains like DH5a 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.*

2.6.3 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 2x 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 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 2x YT or TB medium) because lysis efficiency might be lower when using cell pellets which are too large.

2.7 Elution procedures

See the following table for correlation between the dispensed elution buffer volume and typical recoveries following the standard protocol under vacuum.

The recommended dispense volume of elution buffer is 125 μL .

| Correlation between dispensed elution buffer volume and typical recovery | | | | | |
|--|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|
| Dispensed elution buffer | 75 μL | 100 μL | 125 μL | 150 μL | 175 μL |
| Recovered elution buffer containing PCR-products | 30 \pm 5 μL | 55 \pm 5 μL | 80 \pm 5 μL | 105 \pm 5 μL | 130 \pm 5 μL |

- Recovered DNA, μg
- ◆ Concentration, $\text{ng}/\mu\text{L}$
- ▲ Recovery, %

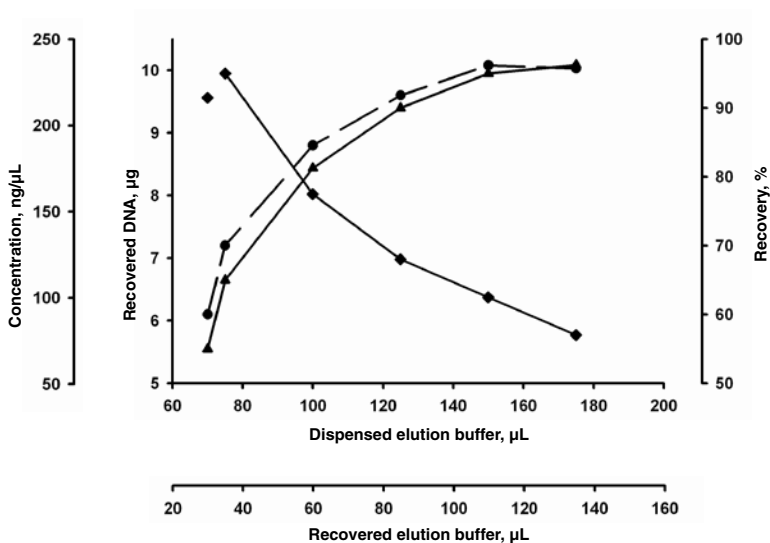


Figure 1: Recovery rate and concentration depend on elution volume.

10 μg of pBluescript plasmid were purified with NucleoSpin[®] 8 Plasmid and eluted with the indicated elution buffer volumes.

3 Storage conditions and preparation of working solutions

Attention: Buffers A3 and AW contain chaotropic salts which are irritant. Buffer A2 contains SDS and sodium hydroxide which are irritant and hazardous. Wear gloves and goggles!

CAUTION: Buffers A3 and AW contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Storage conditions:

- All kit components can be stored at room temperature (18–25 °C) and are stable up to one year. Always keep buffer bottles tightly closed, especially if buffers are preheated during the preparation.
- Sodium dodecyl sulfate (SDS) in Buffer A2 may precipitate if stored at temperatures below 20 °C. If a precipitate is observed in Buffer A2, incubate the bottle at 30–40 °C for several minutes and mix well.

Before starting any **NucleoSpin® 8 Plasmid** protocol, prepare the following:

- Before the first use of the kit, dissolve **RNase A** with 1 mL of **Buffer A1** and vortex. Transfer all of the resulting solution into the Buffer A1 bottle and mix thoroughly. Indicate date of RNase A addition. Store Buffer A1 containing RNase A at 4 °C. The solution will be stable at this temperature for at least six months.
- **Wash Buffer A4:** Add the indicated volume of ethanol (96–100 %) to Buffer A4 Concentrate before use. Mark the label of the bottle to indicate that ethanol was added.

| NucleoSpin® 8 Plasmid | | |
|---------------------------------|------------------------------|---|
| | 12 x 8 preps | 60 x 8 preps |
| REF | 740621 | 740621.5 |
| Wash Buffer A4 (Concentrate) | 100 mL Add 400 mL ethanol | 4 x 100 mL Add 400 mL ethanol to each bottle |







| NucleoSpin® 8 Plasmid Core Kit | |
|---------------------------------|---|
| REF | 48 x 8 preps 740461.4 |
| Wash Buffer A4 (Concentrate) | 2 x 100 mL Add 400 mL ethanol to each bottle |

4 Safety instructions

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

| Component | Hazard contents | GHS symbol | | Hazard phrases | Precaution phrases |
|-----------|--|---|---------------------------|-----------------------|--|
| Inhalt | Gefahrstoff | GHS Symbol | | H-Sätze | P-Sätze |
| A2 | Sodium hydroxide 0.5–2.0 % <i>Natriumhydroxid-Lösung</i> 0.5–2.0 % |  | Warning <i>Achtung</i> | 315, 319 | 280, 302+352, 305+351+338, 332+313, 337+313 |
| A3 | Guanidine hydrochloride 36–50 % <i>Guanidinhydrochlorid</i> 36–50 % |  | Warning <i>Achtung</i> | 302, 319 | 280, 301+312, 305+351+338, 330, 337+313 |
| AW | Guanidine hydrochloride 36–50 % + isopropanol 20–50 % <i>Guanidinhydrochlorid</i> 36–50 % + <i>Isopropanol</i> 20–50 % |   | Warning <i>Achtung</i> | 226, 302, 319, 336 | 210, 233, 280, 301+312, 305+351+338, 330, 337+313, 403+235 |
| RNase A | RNase A, lyophilized <i>RNase A, lyophilisiert</i> |   | Danger <i>Gefahr</i> | 317, 334 | 261, 280, 302+352, 304+340, 333+313, 342+311, 363 |

Hazard phrases

| | |
|-------|--|
| H 226 | Flammable liquid and vapour. <i>Flüssigkeit und Dampf entzündbar.</i> |
| H 302 | Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i> |
| H 315 | Causes skin irritation. <i>Verursacht Hautreizungen.</i> |
| H 317 | May cause an allergic skin reaction. <i>Kann allergische Hautreaktionen verursachen.</i> |
| H 319 | Causes serious eye irritation. <i>Verursacht schwere Augenreizung.</i> |
| H 334 | May cause allergy or asthma symptoms or breathing difficulties if inhaled. <i>Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.</i> |
| H336 | May cause drowsiness or dizziness. <i>Kann Schläfrigkeit und Benommenheit verursachen.</i> |

Precaution phrases

- P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.
- P 233 Keep container tightly closed.
Behälter dicht verschlossen halten.
- P 261 Avoid breathing dust.
Einatmen von Staub vermeiden.
- P 280 Wear protective gloves / eye protection.
Schutzhandschuhe / Augenschutz tragen.
- P 301+312 IF SWALLOWED: Call a POISON CENTER/ doctor/.../if you feel unwell.
BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /... anrufen.
- P 302+352 IF ON SKIN: Wash with plenty of water/...
BEI KONTAKT MIT DER HAUT: Mit viel Wasser/... waschen.
- P 304+340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
Bei Einatmen: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.
- P 305+351+338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.
- P 330 Rinse mouth.
Mund ausspülen.
- P 332+313 If skin irritation occurs: Get medical advice / attention.
Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.
- P 333+313 If skin irritation occurs: Get medical advice / attention.
Bei Hautreizung- oder -ausschlag: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.
- P 337+313 Get medical advice / attention.
Bei anhaltender Augenreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor/...
Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM /Arzt/... anrufen.
- P 363 Wash contaminated clothing before reuse.
Kontaminierte Kleidung vor erneutem Tragen waschen.
- P 403+235 Store in a well ventilated place. Keep cool.
Kühl an einem gut belüfteten Ort aufbewahren.

For further information please see Material Safety Data Sheets (www.mn-net.com).
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (www.mn-net.com).

5 Protocols

5.1 NucleoSpin® 8 Plasmid – manual vacuum processing

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 19–20.
- For detailed information on each step, see page 21.
- For use of the NucleoSpin® 8 Plasmid Core Kit (REF 740461.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer A1 and Buffer A4 were prepared according to section 3.
- Set up the vacuum according to the scheme.

Protocol-at-a-glance

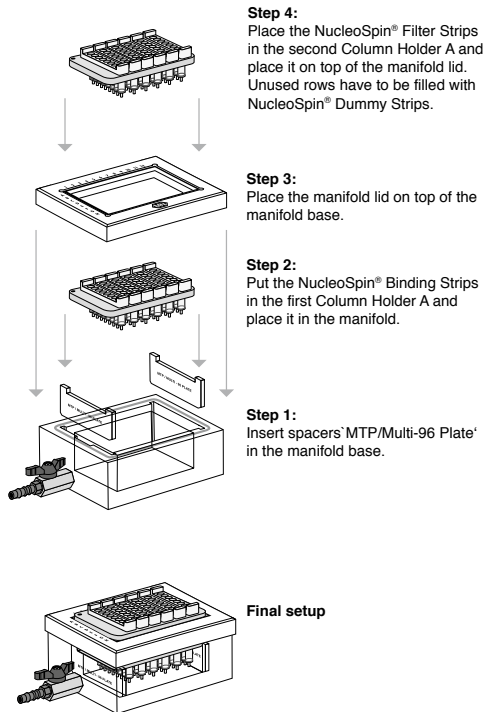
| | | |
|----------|--|---|
| 1 | Cultivate and harvest bacterial cells | 1.5 mL–5 mL LB or up to 2.5 mL 2 x YT or TB |
| | | 10 min, 1,000 x g |
| <hr/> | | |
| 2 | Resuspend bacterial cells | 250 µL A1 |
| | | Mix or shake |
| <hr/> | | |
| 3 | Lyse bacterial cells | 250 µL A2 |
| | | RT, 2–5 min Shake |
| <hr/> | | |
| 4 | Neutralize | 350 µL A3 |
| | | Mix or shake |
| <hr/> | | |
| | | Prepare vacuum manifold for lysate clearing step |
| <hr/> | | |
| 5 | Transfer crude lysates to NucleoSpin® Plasmid Filter Strips (purple rings) | |

- | | | |
|-----------|---|--|
| 6 | <p>Clear crude lysates by vacuum filtration directly into the NucleoSpin® Plasmid Binding Strips (transparent rings)</p> <p><i>Optional: Incubate 1–3 min before applying vacuum</i></p> | <p>-0.2 to -0.4 bar*, 1–5 min</p> |
| <hr/> | | |
| 7 | <p>Reassemble vacuum manifold</p> <p>Discard NucleoSpin® Plasmid Filter Strips</p> <p>Remove NucleoSpin® Plasmid Binding Strips with cleared lysates and insert MN Wash Plate</p> <p>Place NucleoSpin® Plasmid Binding Strips on top of the manifold</p> | |
| <hr/> | | |
| 8 | <p>Bind DNA to silica membrane of the NucleoSpin® Plasmid Binding Strips by applying vacuum</p> | <p>-0.2 to -0.4 bar*, 1 min</p> |
| <hr/> | | |
| 9 | <p>Wash silica membrane</p> | <p><i>(Optional: 600 µL AW)</i></p> <p>900 µL A4</p> <p>900 µL A4</p> <p>-0.2 to -0.4 bar*, 1 min each step</p> |
| <hr/> | | |
| 10 | <p>Remove MN Wash Plate</p> | |
| <hr/> | | |
| 11 | <p>Dry NucleoSpin® Plasmid Binding Strips by applying vacuum</p> <p><i>Optional: Dry the outlets of the NucleoSpin® Plasmid Binding Strip by placing it on a sheet of filter paper before applying vacuum</i></p> | <p>Full vacuum 10–15 min (run pump continuously)*</p> |
| <hr/> | | |
| 12 | <p>Insert Rack of Tube Strips</p> | |
| <hr/> | | |
| 13 | <p>Elute plasmid DNA</p> <p><i>Optional: Incubate 1–3 min</i></p> | <p>75–150 µL AE</p> <p>-0.4 to -0.6 bar*, 1 min</p> |

* Reduction of atmospheric pressure

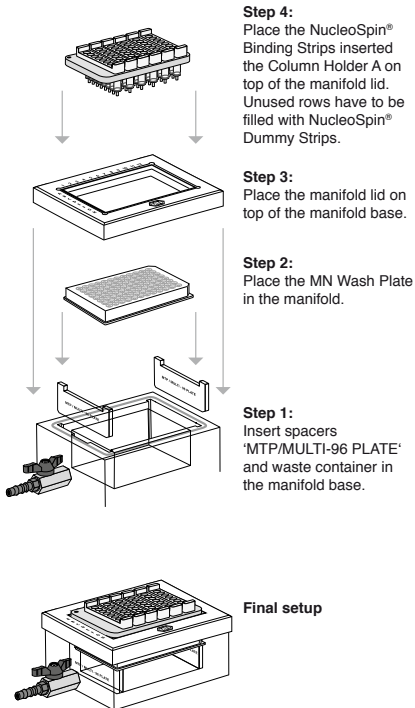
Setup of vacuum manifold: Lysate clearing

Lysate clearing

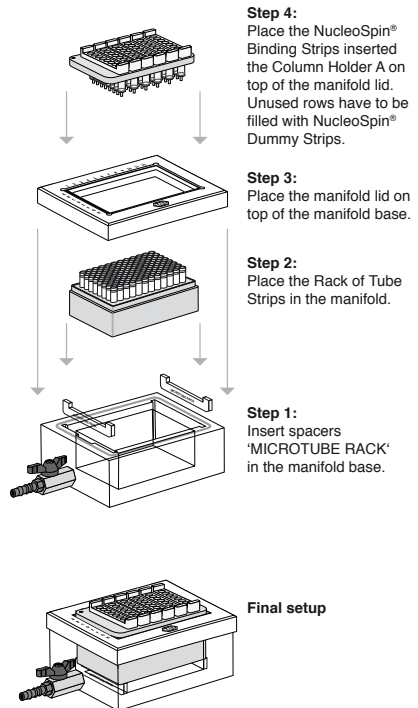


Setup of vacuum manifold: Binding /Washing /Elution steps

Binding / Washing steps



Elution step



Detailed protocol

For processing of **NucleoSpin® 8 Plasmid** under vacuum the NucleoVac 96 Vacuum Manifold and the Starter Kit A are required (see ordering information). Starter Kit A contains the Column Holders A and the NucleoSpin® Dummy Strips to close unused rows.

Before starting the preparation:

- Check if Buffer A1 and Buffer A4 were prepared according to section 3.
 - Prepare column holders: Insert NucleoSpin® Plasmid Binding Strips in the first column holder and NucleoSpin® Plasmid Filter Strips into the second column holder. Close unused wells of each column holder with NucleoSpin® Dummy Strips.
-

1 Cultivate and harvest bacterial cells

Centrifuge the bacteria cultures (1.5–5 mL LB or up to 2.5 mL 2 x YT or TB) for **10 min** at **1,000 x g**.

It is highly recommended centrifuging the bacterial cultures under the above mentioned conditions. 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 clean paper sheet or soft tissue.

Optional: Transfer bacteria cultures grown in tubes to a Square-well Block. Alternatively perform the next three steps in the tubes.

2 Resuspend bacterial cells

Add **250 µL Buffer A1 with RNase A** to each sample. Resuspend the bacterial pellet by vortexing or mixing by pipetting up and down. Resuspend bacterial cells completely. No clumps should be visible.

3 Lyse bacterial cells

Add **250 µL Buffer A2** to the suspension. (For lysis in tubes: close the culture tube and mix by inverting several times.)

Incubate at **room temperature (18–25 °C)** 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** to the suspension. (For lysis in tubes: close the culture tube and mix by inverting several times. For lysis in plates: either mix by pipetting up and down after addition of Buffer A3 or before loading to the NucleoSpin® Plasmid Filter Strips.)

Optional: Incubate on ice for 5 min for optimal formation of precipitate.

Prepare the NucleoVac 96 Vacuum Manifold

Prepare manifold for filtration of crude lysates (see page 19):

Insert spacers labeled 'MTP/Multi-96 Plate' notched side up into the grooves located on the short sides of the manifold. Insert waste container into the center of the manifold base.

Insert an appropriate number of NucleoSpin® Plasmid Binding Strips (transparent rings) in a Column Holder A. Close any unused openings of the Column Holder A with NucleoSpin® Dummy Strips. Place the column holder with the binding strips into the manifold base.

Close the manifold with the manifold lid.

Insert desired number of NucleoSpin® Plasmid Filter Strips (purple rings) in a second Column Holder A. Use NucleoSpin® Dummy Strips to close unused openings.

Place the column holder with the NucleoSpin® Plasmid Filter Strips on top of the manifold.

5 Transfer crude lysates onto the NucleoSpin® Plasmid Filter Strips

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

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

6 Clear crude lysate by vacuum filtration

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

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

* Reduction of atmospheric pressure

7 Reassemble vacuum manifold

Remove and discard the NucleoSpin® Plasmid Filter Strips. Open the manifold lid. Remove the Column Holder A with the NucleoSpin® Plasmid Binding Strips (transparent rings) with cleared lysates.

Insert the MN Wash Plate on the spacers inside the manifold base (see page 20). Close the manifold base with the manifold lid. Place the column holder with the binding strips on top of the manifold.

8 Bind DNA to silica membrane

Apply vacuum of **-0.2 to -0.4 bar* (1 min)**. If necessary, press down the Column Holder A with NucleoSpin® Plasmid Binding Strips slightly until flow-through starts. Adjust vacuum to establish a flow rate of 1–2 drops per second.

When the cleared lysate has drained off, release the vacuum.

9 Wash silica membrane

1st wash (optional)

Add **600 µL of Buffer AW** to each well of the NucleoSpin® Plasmid Binding Strips. Apply vacuum of **-0.2 to -0.4 bar* (1 min)**. If necessary, press down the Column Holder A with NucleoSpin® Plasmid Binding Strips slightly. Allow the buffer to pass the columns.

Release the vacuum.

Note: This additional wash step is recommended if the bacterial host strain has a high endogenous nuclease activity (e.g., E. coli HB 101, BMH 71-18 mutS, JM, or any wild-type strains) or if sequencing results need to be improved.

2nd wash

Add **900 µL Buffer A4** (with ethanol) to each well. Apply vacuum of **-0.2 to -0.4 bar* (1 min)** and allow the buffer to pass the columns.

Release the vacuum.

3rd wash

Repeat the wash step with **900 µL Buffer A4**. Apply vacuum of **-0.2 to -0.4 bar* (1 min)** and allow the buffer to pass the columns.

* Reduction of atmospheric pressure

10 Remove MN Wash Plate

After the final washing step, close the valve, release the vacuum, and remove the Column Holder A with the inserted NucleoSpin® Plasmid Binding Strips. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

11 Dry NucleoSpin® Plasmid Binding Strips

Remove any residual wash buffer from the NucleoSpin® Plasmid Binding Strips. If necessary, tap the outlets of the strips onto a clean blotting paper or soft tissue until no further drops come out.

Close the manifold base with the manifold lid. Place the column holder with the binding strips on top of the manifold.

Apply vacuum of **-0.4 to -0.6 bar*** for **at least 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 A4 is of more importance than reaching the precise mentioned atmospheric pressure.

It is more important to have a continuous air-flow to evaporate the ethanol from Wash Buffer A4.

Note: The ethanol in Buffer A4 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, release the vacuum.

12 Elute into Tube Strips

Remove the manifold lid with the Column Holder A from the vacuum manifold. Insert spacers 'Microtube rack', notched side up, into the grooves located at the short sides of the vacuum manifold.

Insert the Rack of Tube Strips on the spacers inside the manifold base and reinstall the vacuum manifold as described before.

or

Insert Elution Plate U-bottom (not supplied with the kit, see ordering information)

Remove the manifold lid with the Column Holder A from the vacuum manifold. Insert the Elution Plate on the spacers inside the manifold base. Close the manifold base with the manifold lid. Place the column holder with the Binding Strips (transparent rings) on top of the manifold.

* Reduction of atmospheric pressure

For elution into microtiter plates, the spacers 'MTP/Multi-96 Plate' are required which are already inserted into the manifold base from the previous steps.

13 Elute plasmid DNA

Elute the DNA by adding **125 µL Buffer AE** (5 mM Tris-HCl, pH 8.5; 125 µL is recommended, a volume range of 75–150 µL is possible, see section 2.7) or sterile distilled water (pH 7.5–8.5) to each well of the NucleoSpin® Plasmid Binding Strips.

The elution buffer should be dispensed carefully onto the center of the silica membrane. Incubate the buffer on the membrane for **1–3 minutes** at **room temperature**. Apply vacuum of **-0.4 to -0.6 bar*** (1 min). If necessary, press down the column holder slightly and collect the eluted DNA. After the elution buffer has passed the wells, release vacuum.

Remove the Rack of Tube Strips or Elution Plate U-bottom containing eluted DNA and seal the plate/strips with adhesive cover foil or Cap Strips, respectively, for further storage.

* Reduction of atmospheric pressure

5.2 NucleoSpin® 8 Plasmid – elution of DNA using a centrifuge

Elution of purified DNA in a centrifuge can be performed when higher concentrations of the final DNA are required for downstream applications. Using a centrifuge allows reduction of the dispensed volume to 50–75 µL.

Required hardware:

- For centrifugation, a microtiterplate centrifuge that can accommodate the NucleoSpin® Plasmid Binding Strips (inserted into a Column Holder C) stacked on a Rack of Tube Strips is required (bucket height: 85 mm). It is also necessary that the centrifuge reaches accelerations of 5,600–6,000 x g.
- For processing the 8-well strips, the Starter Set C (see ordering information), containing Column Holders C, MN Square-well Blocks, and Rack of Tube Strips is required.

-
- 1 Stop the method after the final washing step with Buffer A4. Remove Column Holder A with inserted NucleoSpin® Plasmid Binding Strips from the manifold's top and tap on a filter paper sheet to remove residual wash buffer from the outlets.

Insert NucleoSpin® Plasmid Binding Strips into a Column Holder C.

-
- 2 Place the Column Holder C with the binding strips on top of a MN Square-well Block (not included in the kits, see ordering information) and centrifuge for **10 min** at **maximum speed** (> 4,000 x g, optimal 5,800 x g).

Note: Do not use a microtiter plate as a support for the NucleoSpin® Plasmid Binding Strips. Microtiter plates may crack under centrifugation at > 1,500 x g.

-
- 3 Place the Column Holder C with inserted NucleoSpin® Binding Strips on top of a Rack of Tube Strips. Dispense **Elution Buffer AE** (50–150 µL) directly onto the silica membrane. Incubate for **1–3 min** at **room temperature**.

Note: Do not use a microtiter plate as elution plate. Microtiter plates may crack under centrifuging at > 1,500 x g. Alternatively, a 96-well PCR plate can be inserted into the Square-well Block for elution.

-
- 4 Centrifuge for **2 min** at **maximum speed** (> 4,000 x g, optimal 5,800 x g) to collect the plasmid DNA.

Remove the Rack of Tube Strips containing eluted DNA and close them with Cap Strips for further storage.

6 Appendix

6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|-------------------------------------|---|
| | <p><i>Cell pellet not properly resuspended</i></p> <ul style="list-style-type: none">It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of Lysis Buffer A2. If necessary, increase number of mixing cycles or duration of shaking. |
| Incomplete lysis of bacterial cells | <p><i>SDS in Buffer A2 precipitated</i></p> <ul style="list-style-type: none">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 5 min and mix well before use. <p><i>Too many bacterial cells used</i></p> <ul style="list-style-type: none">Usage of LB as the growth medium is recommended. When using rich media like TB, cultures reach very high cell densities. Reduce culture volume to 1.0–1.5 mL. |
| | <p><i>No or not enough antibiotic used during cultivation</i></p> <ul style="list-style-type: none">Cells harboring the plasmid of interest may become overgrown by non-transformed cells. Add appropriate amounts of freshly prepared stock solutions of antibiotic to all media. |
| | <p><i>Overgrown bacterial cultures</i></p> <ul style="list-style-type: none">See suggestions in section 2.6 ‘Growth of bacterial cultures’. |
| Poor plasmid yield | <p><i>High-copy number plasmid was not used</i></p> <ul style="list-style-type: none">Use high-copy number plasmid. <p><i>Incomplete lysis of bacterial cells</i></p> <ul style="list-style-type: none">See ‘Possible cause and suggestions’ above. <p><i>No ethanol was added to Buffer A4 Concentrate, ethanol evaporated</i></p> <ul style="list-style-type: none">Add indicated volume of ethanol to Buffer A4 Concentrate and mix. Keep bottle tightly closed to prevent evaporation of ethanol. |

| Problem | Possible cause and suggestions |
|---------|--------------------------------|
|---------|--------------------------------|

Poor plasmid yield
(continued)

Elution conditions are not optimal

- If possible, use a slightly alkaline elution buffer like Buffer AE (5 mM Tris-HCl, pH 8.5). When using nuclease-free water for elution, make sure the pH value is within the range of pH 8.0–8.5. Elution efficiencies drop drastically at pH < 7.

Excessive mixing steps

- Reduce number of mixing cycles, reduce shaker action after addition of Lysis Buffer A2 and Neutralization Buffer A3 or before transfer of crude lysate to the NucleoSpin® Plasmid Filter Strips. Mixing will cause shearing of chromosomal DNA, leading to a co-purification during the preparation of plasmid DNA.

Culture volume was too high

- Reduce culture volume if lysate is too viscous for gentle and complete mixing.

Contamination with chromosomal DNA

Bacterial culture overgrown

- Overgrown bacterial cultures contain lysed cells and degraded DNA. See suggestions in section 2.6 'Growth of bacterial cultures'.

Lysis was too long

- Lysis step must not exceed 5 min.

Tips

- Use wide bore disposable tips for transfer of crude lysate to the NucleoSpin® Plasmid Filter Strips to prevent shearing of chromosomal DNA.

RNA in the eluate

RNA was not degraded completely

- Ensure that RNase A was added to Buffer A1 before use.
- Reduce culture volume if necessary.

| Problem | Possible cause and suggestions |
|----------------|---------------------------------------|
|----------------|---------------------------------------|

Carry-over of ethanol, inhibition of downstream analysis, or problems with sample loading onto agarose

- Be sure to remove all of ethanolic Buffer A4 after the final washing step. Dry the NucleoSpin® Plasmid Binding Strips for at least 10 min with maximum vacuum.

Suboptimal performance of plasmid DNA in sequencing reactions, problems with downstream applications

Elution of plasmid DNA with TE buffer

- EDTA may inhibit enzymatic reactions like DNA sequencing. Repurify the plasmid DNA and elute with Buffer AE or nuclease-free water. Alternatively, the plasmid DNA may be precipitated with ethanol, and redissolved in Buffer AE or nuclease-free water.

E. coli strains with high endogenous-nuclease levels are used as host

- Perform the washing step with Buffer AW before washing with ethanolic Buffer A4.

Not enough DNA used for sequencing reactions

- Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions.
-

6.2 Ordering information

| Product | REF | Pack of |
|---|------------|----------------|
| NucleoSpin® 8 Plasmid | 740621 | 12 x 8 preps |
| | 740621 .5 | 60 x 8 preps |
| NucleoSpin® 8 Plasmid Core Kit | 740461 .4 | 48 x 8 preps |
| NucleoSpin® 96 Plasmid | 740625 .1 | 1 x 96 preps |
| | 740625 .4 | 4 x 96 preps |
| | 740625 .24 | 24 x 96 preps |
| NucleoSpin® 96 Plasmid Core Kit | 740616 .4 | 4 x 96 preps |
| | 740616 .24 | 24 x 96 preps |
| Buffer A1 (without RNase A) | 740911 .1 | 1 L |
| Buffer A2 | 740912 .1 | 1 L |
| Buffer A3 | 740913 .1 | 1 L |
| Buffer A4 Concentrate (for 1 L Buffer A4) | 740914 .1 | 200 mL |
| Buffer AW | 740916 .1 | 1 L |
| Buffer AE | 740917 .1 | 1 L |
| RNase A (lyophilized) | 740505 | 100 mg |
| NucleoVac 96 Vacuum Manifold | 740681 | 1 |
| NucleoVac Vacuum Regulator | 740641 | 1 |
| Starter Set A (for processing NucleoSpin® 8-well strips on NucleoVac 96 Vacuum Manifold) | 740682 | 1 |
| Starter Set C (for processing NucleoSpin® 8-well strips under centrifugation) | 740684 | 1 |
| Round-well Block with Cap Strips | 740475 | 4 sets |
| | 740475 .24 | 24 sets |

| Product | REF | Pack of |
|--|----------------------|-------------------|
| 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 | 48 288 |
| MN Square-well Block | 740476 740476 .24 | 4 24 |
| MN Wash Plate | 740479 740479 .24 | 4 24 |
| 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 |
| Self-adhering Foil | 740676 | 50 |

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

6.3 Product use restriction/warranty

NucleoSpin® 8 Plasmid (Core Kit) components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

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MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 (0) 24 21 969 270
e-mail: tech-bio@mn-net.com

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