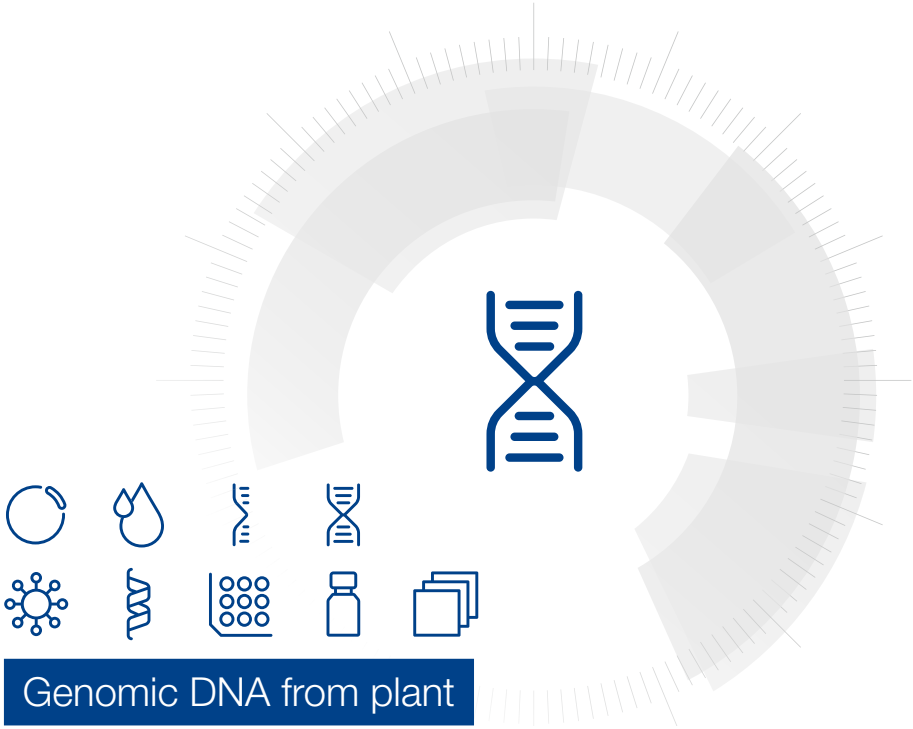
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from plant

- NucleoSpin® 96 Plant II
- NucleoSpin® 96 Plant II Core Kit

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1 Components

1.1 Kit contents

	NucleoSpin® 96 Plant II		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**
REF	**740663.2**	**740663.4**	**740663.24[1]**
Lysis Buffer PL1	125 mL	250 mL	6 x 250 mL
Lysis Buffer PL2[2]	100 mL	200 mL	6 x 200 mL
Precipitation Buffer PL3	25 mL	50 mL	6 x 50 mL
Binding Buffer PC	125 mL	250 mL	6 x 250 mL
Wash Buffer PW1	100 mL	2 x 100 mL	12 x 100 mL
Wash Buffer PW2 (Concentrate)[2]	100 mL	2 x 100 mL	12 x 100 mL
Elution Buffer PE[3]	60 mL	125 mL	6 x 125 mL
RNase A (lyophilized)[2]	30 mg	2 x 30 mg	12 x 30 mg
NucleoSpin® Plant II Binding Plate (dark green rings)	2	4	24
MN Wash Plate	2	4	24
Rack of Tube Strips[4] (for lysis and elution)	4	8	32
Cap Strips	24	48	288
MN Square-well Block	6	12	72
Gas-permeable Foil	10	20	120
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[1] The kit for 24 x 96 preparations REF 740663.24 consists of 6 x REF 740663.4.

[2] For preparation of working solutions and storage conditions see section 3.

[3] Composition of Elution Buffer PE: 5 mM Tris/HCl, pH 8.5

[4] 1 rack = 12 strips with 8 tubes each, Cap Strips included

Kit contents continued

	NucleoSpin® 96 Plant II Core Kit
	4 x 96 preps
REF	740468.4
Lysis Buffer PL1	250 mL
Lysis Buffer PL2[1]	200 mL
Precipitation Buffer PL3	50 mL
Binding Buffer PC	250 mL
Wash Buffer PW1	2 x 100 mL
Wash Buffer PW2 (Concentrate)[1]	2 x 100 mL
Elution Buffer PE[2]	125 mL
RNase A (lyophilized)[1]	2 x 30 mg
NucleoSpin® Plant II Binding Plate (dark green rings)	4
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1.2 Reagents to be supplied by user

- 96–100 % ethanol

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.

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

[2] Composition of Elution Buffer PE: 5 mM Tris/HCl, pH 8.5

2 Product description

2.1 The basic principle

The **NucleoSpin® 96 Plant II** kit is designed for the isolation of genomic DNA from plant materials. After the plant samples have been homogenized, the DNA can be extracted with lysis buffers containing chaotropic salts, denaturing agents and detergents. The standard isolation ensures the lysis of plant material with the CTAB Lysis Buffer PL1, which is specially developed for plants. In addition, an SDS based lysis buffer, Buffer PL2, is provided as an alternative. Buffer PL2 requires subsequent protein precipitation with potassium acetate. Lysates should be cleared by centrifugation in order to remove polysaccharides, contaminations, and residual cellular debris. The clear supernatant is mixed with Binding Buffer PC to create conditions for optimal binding to the silica membrane in the binding plate. After washing with two different buffers (Buffer PW1 and Buffer PW2) DNA can be eluted in low salt Buffer PE or water and is ready to use for subsequent analysis and processing.

2.2 Kit specifications

- **NucleoSpin® 96 Plant II** is designed for the isolation of genomic DNA from plant material.
- **NucleoSpin® 96 Plant II** allows parallel purification of multiples of 96 samples each with up to 100 mg sample per well (wet weight).
- Depending on the individual sample, **NucleoSpin® 96 Plant II** shows yields in the range of 1–30 µg DNA (maximum column capacity is about 30 µg) with an A_{260}/A_{280} ratio between 1.80 and 1.90 and typical concentrations of 100–200 ng/µL. The amount of DNA that can be expected per mg of sample extracted depends on the size and ploidy of the genome. For example, 100 mg fresh wheat with a hexaploid genome (1.7×10^{10} bp) contain 30 µg DNA, whereas the same amount of Arabidopsis with a smaller diploid genome (1.9×10^{8} bp) yields only 3 µg DNA.
- The eluted DNA is ready to use in subsequent reactions like PCR, restriction analysis, etc.
- **NucleoSpin® 96 Plant II** can be processed under vacuum, positive pressure or in a centrifuge.
- Two lysis buffers, based on CTAB (PL1) or SDS (PL2) are provided.
- **NucleoSpin® 96 Plant II** can be used manually with the NucleoVac 96 Vacuum Manifold (see ordering information) or other vacuum devices.

Kit specifications at a glance

Parameter	NucleoSpin® 96 Plant II
Technology	Silica membrane technology
Format	96-well plates
Processing	Manual or automated, vacuum, positive pressure or centrifugation

Kit specifications at a glance	
Use	For research use only
Sample material	20–100 mg plant tissue, plant cells (wet weight)
Fragment size	50 bp–approx. 50 kpb
Typical yield	1–30 µg
A_{260}/A_{280}	1.8–1.9
Elution volume	100–200 µL
Preparation time	60 min/plate (excl. lysis)
Binding capacity	30 µg

2.3 Required hardware

NucleoSpin® 96 Plant II can be processed under vacuum or with centrifugation. Certain hardware for processing is required.

Centrifugation

For centrifugation, a microtiterplate centrifuge is required. This centrifuge must be able to accomodate the NucleoSpin® Plant II Binding Plate stacked on a Round- or Square-well Block and reach accelerations of 5,600–6,000 x *g* is required (bucket height: 85 mm).

Regarding waste collection, suitable consumables (e.g., MN Square-well Blocks) are necessary and they are not included in the kit. For the most convenient handling, without the need of emptying and reusing the MN Square-well Blocks, we recommend using six MN Square-well Blocks if two 96-well plates are processed at once (see ordering information). Alternatively, it is possible to empty the MN Square-well Blocks after every centrifugation step, reducing the amount of MN Square-well Blocks needed.

Vacuum processing

The **NucleoSpin® 96 Plant II** kit can be used with the NucleoVac 96 Vacuum Manifold (see ordering information). When using **NucleoSpin® 96 Plant II** with less than 96 samples, Self adhering PE Foil (see ordering information) should be used in order to close and protect non-used wells of the NucleoSpin® Plant II Binding Plate and thus guarantee proper vacuum.

Establish a reliable vacuum source for the NucleoVac 96 Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of -0.2 to -0.4 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information) is recommended. Alternatively, adjust the vacuum so that during the purification the sample flows through the column with a rate of 1–2 drops per second. Depending on the amount of sample being used, the vacuum times may need to be increased for complete filtration.

Additionally, a suitable centrifuge for sample preparation steps may be required.

For general consumables and equipment needed, please see section 1.2.

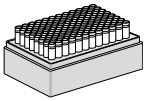
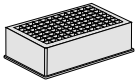
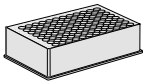
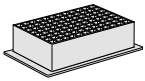
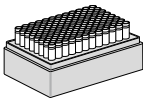
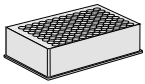
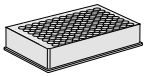
2.4 Recommended accessories for use of the NucleoSpin® 96 Plant II Core Kit

The **NucleoSpin® 96 Plant II Core Kit** provides all necessary buffers, enzymes and NucleoSpin® Binding Plates. Accessories (e.g., lysis plates, waste collection plates, elution plates or tubes) are not provided with the Core Kit. The reduced kit composition along with a large variety of separately available accessories, allow optimal adjustment of the kit to individual user needs. The user can select additional consumables according to his requirements for highest flexibility.

The **NucleoSpin® 96 Plant II Core Kit** provides buffers RNase A, and NucleoSpin® Binding Plates only. Accessory plates (e.g., elution plates) are not provided with the core kit. The user can individually select additional consumables from a variety of suitable accessory plates according to his requirements for highest flexibility.

For use of **NucleoSpin® 96 Plant II Core Kit** follow the standard protocols (see section 5.1or 5.2, respectively).

Recommended accessories for use of the **NucleoSpin® 96 Plant II Core Kit** are available from MACHEREY-NAGEL. For ordering information please refer to section 6.2.

		Suitable consumables, not supplied with the core kits		Remarks
1.	Homogenize samples	Rack of Tube Strips with Cap Strips		
4.	Adjust binding conditions	Square-well Block or		For mixing cleared lysate with Buffer PC
		Round-well Block or		
		MN Square-well Block		
7.	Wash silica membrane	MN Wash Plates		MN Wash Plate minimizes the risk of cross contamination (vacuum processing)
8.	Elute DNA	Rack of Tubes Strips with Cap Strips or	 	
		Round-well Block or		
		Round-well Block Low (centrifugation only)		

2.5 Automated processing on robotic platforms

NucleoSpin® 96 Plant II can be used fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 96 Plant II** on a certain workstation please contact MN. Full processing under vacuum enables complete automation without the need for centrifugation steps for drying of the binding membrane or for elution.

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® Plant II Binding Plate.

Drying of the NucleoSpin® Plant II Binding Plates under vacuum is sufficient because the bottom of the plate is protected by the MN Wash Plate during the washing steps. As a result, it is recommended to integrate the MN Wash Plate into the automated procedure to protect against these wash buffer residues. 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. In addition, thorough cleaning of the vacuum chamber is recommended after each run to prevent forming of gDNA-containing aerosols.

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® 96 Plant II** kit on various liquid handling instruments can also be found at ***www.mn-net.com*** at Bioanalysis / Literature.

2.6 Storage and homogenization of samples

We recommend using young plant samples and keeping the plants in the dark for about 12 h before collecting samples (if possible) in order to reduce the polysaccharide content.

Plant samples can be stored frozen, under ethanol, or lyophilized. In many cases lyophilized, dried material can be processed more easily and gives higher yield. However, keep in mind that dried samples may reduce the amount of starting material by the factor 5 (for example, 20 mg dried plant leaves vs.100 mg fresh weight).

As plant tissue is very robust, the lysis procedure is most effective with well homogenized, powdered samples. Suitable methods include grinding with pestle and mortar in the presence of liquid nitrogen or using steel beads. We also recommend the use of other commercial homogenizers, bead mills, etc.

Methods to homogenize samples

- Commercial homogenizers, for example Crush Express for 96-well homogenization (Saaten-Union Resistenzlabor GmbH, D-33818 Leopoldshöhe), Tissue Striker (www.KisanBiotech.com), or Geno/Grinder 2000 can be used.
- Samples can be disrupted using bead based homogenization tools, for example, GenoGrinder (http://www.spexcsp.com or for Germany www.c3-analysentechnik.de) or Mixer Mill MM400 (http://www.retsch.com/products/milling/ball-mills/mm-400/). Please refer to instrument manufacturers recommendations for suitable plates or tubes for homogenization.
- Homogenizing samples by VA steel beads (diameter: 3 mm): Put 4–5 beads and plant material together into a 15 ml plastic tube (Falcon), chill the tube in liquid nitrogen, and vortex for about 30 seconds (e.g., with a Multi Pulse Vortexer, Schütt Labortechnik GmbH, Postfach 3454, D-37024 Göttingen, Germany). Repeat this chilling and

vortexing procedure until the entire plant material is ground to a powder. Chill the tube once more and remove the beads by rolling them out gently or remove them with a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube! This leads to sticking and loss of plant material attached to the beads. Alternativeley, prefilled and ready to use MN Bead Tubes Type G (5 mm steel beads) allow an easy and convenient sample homogenization procedure.

- High throughput homogenization: Add the plant tissue to the individual tubes of the Tube Strips. Add one 3 mm stainless steel bead to each tube and close the individual tubes with Cap Strips. Freeze the sample in liquid nitrogen and insert the Rack of Tube Strips in a suitable homogenization tool (e.g., mixer mill). For disruption, shake the samples for 60–90 s at 30 Hz or until a homogenous plant powder has been formed. If necessary, repeat shaking once. Fresh plant material can also be homogenized with lysis buffer, however, homogenization of fresh plant material with lysis buffer may cause shearing of DNA. For frozen plant material thawing should be avoided during the homogenization. Samples should be frozen in liquid nitrogen before homogenization. Lyophilized or silica-gel dried material can be homogenized with or without lysis buffer. Homogenization of lyophilized tissue with lysis buffer may result in higher yield but also may cause shearing of DNA. Alternativeley, prefilled and ready to use MN Bead Plate Type D allows an easy and convenient sample homogenization procedure.

2.7 Elution procedures

It is possible to adjust the elution method and the volume of the elution buffer to the specific application of interest. In addition to the standard method (recovery rate about 80–90 %) described in the protocols, there are 3 modifications possible:

- High yields: 90–100 % of bound nucleic acids can be eluted by performing two elution steps with volumes as indicated in the protocol, for example 2 x 100 µL. Finally, combine eluates and measure yield.
- Alternatively, use preheated Elution Buffer PE (70 °C): Preheat elution buffer to increase yield. After loading half of the preheated elution buffer (50 µL) onto the membrane, incubate the NucleoSpin® Plant II Binding Plate for 3 min at 60–70 °C. Centrifuge for elution as indicated. Repeat the elution step once.
- Highly concentrated eluates: Using a minimal elution volume (about 50 µL) about 70–80 % of bound nucleic acids can be eluted, resulting in highly concentrated eluates.

Elution may also be performed with Tris-EDTA-buffer (TE) with a pH equal or higher than 8. This will increase DNA stability during long term or multi-use storage at 4 °C (or ambient temperature) by inhibiting omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications.

For optimal performance of isolated DNA in downstream applications, we recommend eluting with the supplied elution buffer and storing it, especially long term, at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

Performance of long-range PCR (e.g., > 10 kbp), or the detection limit of trace amounts of DNA species, may be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at 4 °C or room temperature. This is due to shearing of DNA or adsorption to surfaces.

Due to the dead volume of the silica membrane please note that the difference between the dispensed elution volume and the recovered elution buffer is approximately 45 µL (recovered elution volume = dispensed elution volume - 45 µL).

3 Storage conditions and preparation of working solutions

Attention: Buffer PL1 contains CTAB, Buffer PL2 contains SDS, Buffers PC and PW1 contain chaotropic salt! Wear gloves and goggles!

CAUTION: Buffers PC and PW1 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.

- Store **RNase A** at 4 °C on arrival (storage at 4 °C may cause precipitation of salts in different buffers).
- All other components can be stored at 15–25 °C and are stable until: see package label.

Before starting any **NucleoSpin® 96 Plant II** protocol prepare the following:

- **Lysis Buffer PL2:** Check for precipitated SDS especially after storage at temperatures below 20 °C. If necessary incubate the bottle for several minutes at 30–40 °C and mix well until the precipitate is redissolved completely.
- **Wash Buffer PW2:** Add the indicated volume of ethanol (96–100 %) to Buffer PW2 Concentrate before first use. Store Buffer PW2 at 15–25 °C for up to one year.
- **RNase A:** Add the given volume of water (indicated on the vial, see below) to lyophilized RNase A. Store the RNase A solution at 4 °C for up to 3 months. For longer storage (up to 1 year), the RNase A solution should be divided into small aliquots and stored at -20 °C.

	NucleoSpin® 96 Plant II	**NucleoSpin® 96 Plant II**	**NucleoSpin® 96 Plant II**	**NucleoSpin® 96 Plant II Core Kit**
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**	**4 x 96 preps**
REF	**740663.2**	**740663.4**	**740663.24**	**740468.4**
Wash Buffer PW2 (Concentrate)	100 mL Add 400 mL ethanol	2 x 100 mL Add 400 mL ethanol to each bottle	12 x 100 mL Add 400 mL ethanol to each bottle	2 x 100 mL Add 400 mL ethanol to each bottle
RNase A (lyophilized)	30 mg Add 2.5 mL H_2O	2 x 30 mg Add 2.5 mL H_2O to each vial	12 x 30 mg Add 2.5 mL H_2O to each vial	2 x 30 mg Add 2.5 mL H_2O to each vial

4 Safety instructions

When working with the N**ucleoSpin® 96 Plant II** or **NucleoSpin® 96 Plant II Core** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at http://***www.mn-net.com/msds***).

Caution: Guanidin hydrochloride in Buffer PC and Buffer PW1 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 Plant II** or **NucleoSpin® 96 Plant II Core** 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 NucleoSpin® 96 Plant II – centrifuge processing

- For hardware requirements, refer to section 2.3
- For detailed information on each step, see page 16.
- For use of the NucleoSpin® 96 Plant II Core Kit (REF 740468.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer PW2 and RNase A were prepared according to section 3.
- Set incubator or oven to 65 °C.
- Equilibrate Buffer PE to 70 °C.

Protocol at a glance

1	**Homogenize** samples	**Up to 100 mg wet or 20 mg lyophilized plant tissue** **5,600–6,000 x *g*, 2 min**
2a	**Cell lysis** using Buffer **PL1**	**500 µL PL1** **10 µL RNase A** **Mix** **65 °C, 30 min** **Proceed with step 3**
2b	**Cell lysis** using Buffer **PL2 and PL3**	**400 µL PL2** **10 µL RNase A** **Mix** **65 °C, 30 min** **100 µL PL3** **Mix and incubate on ice for 5 min** **Proceed with step 3**
3	**Clear lysate** by centrifugation	**5,600–6,000 x *g*, 20 min**
4	**Adjust** binding conditions	**Mix 450 µL PC with 400 µL cleared lysate**
5	**Transfer** lysate to NucleoSpin® Plant II Binding Plate	

6	**Bind** DNA to silica membrane of the NucleoSpin® Plant II Binding Plate	**5,600–6,000 x *g*, 2 min**
7	**Wash** and **dry** silica membrane	**400 µL PW1** **5,600–6,000 x *g*, 2 min** **700 µL PW2** **5,600–6,000 x *g*, 2 min** **700 µL PW2** **5,600–6,000 x *g*, 10 min**
8	**Elute** DNA	**100 µL PE (70 °C) (incubate 2 min)** **5,600–6,000 x *g*, 2 min** **Repeat once**

Detailed protocol

- For hardware requirements, refer to section 2.3.
- For use of the NucleoSpin® 96 Plant II Core Kit (REF 740468.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer PW2 and RNase A were prepared according to section 3.
- Set incubator or oven to 65 °C.
- Equilibrate Buffer PE to 70 °C.

1 Homogenize samples

Fill **up to 100 mg wet plant tissue** (or up to **20 mg dried material**, for example lyophilized plant tissue) into each tube of the Tube Strips. Add one 3 mm diameter steel bead to each tube. Close the tubes with Cap Strips. Freeze samples in liquid nitrogen. Disrupt cells by vigorous shaking using a mixer mill. Centrifuge at **5,600 x *g*** for **2 min** and remove Cap Strips.

For further processing use either Buffer PL1 (2 a) or Buffers PL2 / PL3 (2 b)!

2a Cell lysis using Buffer PL1

Add **500 µL Buffer PL1** and **10 µL RNase A** to each sample. Close tubes again using new Cap Strips (supplied). Mix by vigorous **shaking** for **15–30 s. Spin briefly** for **30 s** at **1,500 x *g*** to collect any sample from the Cap Strips. Incubate samples at **65 °C** for **30 min**.

Depending on plant sample and available methods, Buffer PL1 and RNase A may be added to the plant material before homogenization by the appropriate mechanical method.

Proceed with step 3.

2b Cell lysis using Buffer PL2 and PL3

Add **400 µL Buffer PL2** and **10 µL RNase A** to each sample. Close tubes again using new Cap Strips (supplied). Mix by vigorous **shaking** for **15–30 s**. Spin briefly for **30 s** at **1,500 x *g*** to collect any sample from the Cap Strips. Incubate samples at **65 °C** for **30 min**.

Depending on plant sample and available methods, Buffer PL2 and RNase A may be added to the plant material before homogenization by the appropriate mechanical method.

Open tubes, add **100 µL Buffer PL3**, close tubes, mix thoroughly, and incubate for 5 min on ice to precipitate SDS completely.

3 Clear lysate by centrifugation

Centrifuge the samples for **20 min** at **full speed (5,600–6,000 x *g*)**. Remove Cap Strips.

4 Adjust binding conditions

Predispense **450 µL Binding Buffer PC** to each well of a MN Square-well Block. Add **400 µL cleared lysate** of each sample and mix by repeated pipetting up and down. Mix at least 3 times.

5 Transfer lysate to NucleoSpin® Plant II Binding Plate

Place NucleoSpin® Plant II Binding Plate on a MN Square-well Block. Transfer samples from the previous step into the wells of the NucleoSpin® Plant II Binding Plate. Do not moisten the rims of the individual wells while dispensing the samples.

Optional: Seal openings of the binding plate with a Gas-permeable Foil.

6 Bind DNA to silica membrane

Place the NucleoSpin® Plant II Binding Plate stacked on an MN Square-well Block in the rotor buckets. Centrifuge at **5,600–6,000 x *g*** for 5 min.

Typically, lysates will pass through the columns within 1 min. The centrifugation process can be extended to 20 min, if the lysates have not passed completely.

7 Wash silica membrane

1st wash

Add **400 µL PW1** to each well of the NucleoSpin® Plant II Binding Plate.

Seal plate with a Gas-permeable Foil and centrifuge again at **5,600–6,000 x *g*** for 2 min. Place NucleoSpin® Plant II Binding Plate on a new MN Square-well Block.

2nd wash

Add **700 µL PW2** to each well of the NucleoSpin® Plant II Binding Plate.

Seal plate with a Gas-permeable Foil and centrifuge again at **5,600–6,000 x *g*** for **2 min**.

3rd wash

Add **700 µL PW2** to each well of the NucleoSpin® Plant II Binding Plate.

Seal plate with a Gas-permeable Foil and centrifuge again at **5,600–6,000 x *g*** for 10 min. Place NucleoSpin® Plant II Binding Plate on a new MN Square-well Block.

Note: For critical ethanol-sensitive applications, it is recommended to prolong the centrifugation time up to 15 min or incubate at higher temperature. Remove the adhesive foil and place the NucleoSpin® Plant II Binding Plate into an incubator for 20 min at 37 °C to evaporate residual ethanol.

8 Elute DNA

Place NucleoSpin® Plant II Binding Plate on the Rack of Tube Strips. Dispense **100 µL preheated Buffer PE (70 °C)** to each well of the NucleoSpin® Plant II Binding Plate. Dispense the buffer directly onto the membrane.

Optional: Incubate for 2 min at 70 °C before centrifugation.

Centrifuge at **5,600–6,000 x *g*** for 2 min. Remove the NucleoSpin® Plant II Binding Plate from the Rack of Tube Strips.

For optimal yield it is recommended to repeat this step once (incubation of Buffer PE on the membrane not required).

Yields will be 10–20 % higher when eluting with 2 x 100 µL Buffer PE depending on the total amount of DNA. However, the concentration of DNA will be much lower than with 100 µL.

Note: Elution can be done with TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH ≤ 8.0.

5.2 NucleoSpin® 96 Plant II – vacuum processing

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 22.
- For detailed information on each step, see page 22.
- For use of the NucleoSpin® 96 Plant II Core Kit (REF 740468.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer PW2 and RNase A were prepared according to section 3.
- Set incubator or oven to 65 °C.
- Equilibrate Buffer PE to 70 °C.

Protocol at a glance

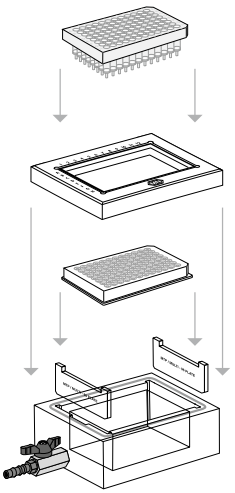
1	**Homogenize** samples	**Up to 100 mg wet or 20 mg lyophilized plant tissue** **5,600–6,000 x *g*, 2 min**
2a	**Cell lysis** using Buffer **PL1**	**500 µL PL1** **10 µL RNase A** **Mix** **65 °C, 30 min** **Proceed with step 3**
2b	**Cell lysis** using Buffer **PL2 and PL3**	**400 µL PL2** **Mix** **65 °C, 30 min** **100 µL PL3** **Mix and incubate on ice for 5 min** **Proceed with step 3**
3	**Clear lysate** by centrifugation	**5,000–6,000 x *g*, 20 min**
4	**Adjust** binding conditions	**Mix 450 µL PC with 400 µL cleared lysate**
5	**Transfer** lysate to NucleoSpin® Plant II Binding Plate	

6	**Bind** DNA to silica membrane of the NucleoSpin® Plant II Binding Plate	**-0.2 to -0.4 bar*** **(2 min)**
7	**Wash** and **dry** silica membrane	**400 µL PW1**
		700 µL PW2
		700 µL PW2–0.4 bar*
		(1 min each step)
		Remove MN Wash Plate
		Dry silica membrane (10 min, maximum vacuum)
8	**Elute** DNA	**100 µL PE (incubate 2 min)**
		-0.4 bar* (2 min)
		Repeat once

* Reduction of atmospheric pressure

Setup of vacuum manifold:

Binding / Washing steps

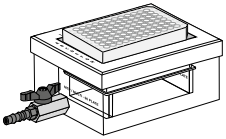


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

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

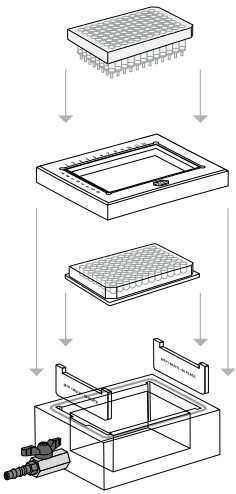
Step 2:
Place the MN Wash Plate in the manifold.

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



Final setup

Elution step

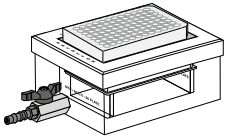


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

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

Step 2:
Place the Elution Plate in the manifold.

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



Final setup

Detailed protocol

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 22.
- For use of the NucleoSpin® 96 Plant II Core Kit (REF 740468.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer PW2 and RNase A were prepared according to section 3.
- Set incubator or oven to 65 °C.
- Equilibrate Buffer PE to 70 °C.
- For detailed information regarding the vacuum manifold set-up see page 22.

1 Homogenize samples

Fill **up to 100 mg wet plant tissue** (or up to **20 mg dried, for example lyophilized, plant tissue**) into each tube of the Tube Strips. Add one 3 mm diameter steel bead to each tube. Close the tubes with Cap Strips. Freeze samples in liquid nitrogen. Disrupt cells by vigorous shaking using a mixer mill. Spin at **5,600 x *g*** for **2 min** and remove Cap Strips.

For further processing use either Buffer PL1 (2 a) or Buffers PL2 / PL3 (2 b)!

2a Cell lysis using Buffer PL1

Add **500 µL Buffer PL1** and **10 µL RNase A** to each sample. Close tubes again using new Cap Strips (supplied). Mix by vigorous **shaking** for **15–30 s. Spin briefly** for **30 s** at **1,500 x *g*** to collect any sample from the Cap Strips. Incubate samples at **65 °C** for **30 min**.

Depending on plant sample and available methods, Buffer PL1 and RNase A may be added to the plant material before homogenization by the appropriate mechanical method.

Proceed with step 3.

2b Cell lysis using Buffer PL2 and PL3

Add **400 µL Buffer PL2** and **10 µL RNase A** to each sample. Close tubes again using new Cap Strips (supplied). Mix by vigorous **shaking** for **15–30 s. Spin briefly** for **30 s** at **1,500 x *g*** to collect any sample from the Cap Strips. Incubate samples at **65 °C** for **30 min**.

Depending on plant sample and available methods, Buffer PL2 and RNase A may be added to the plant material before homogenization by the appropriate mechanical method.

Open tubes, add **100 µL Buffer PL3**, close tubes, mix thoroughly, and incubate for 5 min on ice to precipitate SDS completely.

3 Clear lysate by centrifugation

Centrifuge the samples for **20 min** at **full speed (5,600–6,000 x *g*)**. Remove Cap Strips.

4 Adjust binding conditions

Predispense **450 µL Binding Buffer PC** to each well of a MN Square-well Block. Add **400 µL cleared lysate** of each sample and mix by repeated pipetting up and down. Mix at least 3 times.

5 Transfer lysate to NucleoSpin® Plant II Binding Plate

Place waste tray into manifold base. Insert spacers labeled 'MTP/MULTI-96 PLATE' notched side up into NucleoVac and place the MN Wash Plate on them. Close manifold and place NucleoSpin® Plant II Binding Plate on top of the manifold.

Transfer samples from the previous step into the wells of the NucleoSpin® Plant II Binding Plate. Do not moisten the rims of the individual wells while dispensing the samples.

6 Bind DNA to silica membrane

Apply vacuum of **-0.2 to -0.4 bar*** to allow samples to pass through the membrane. Flowthrough rate should be about 1–2 drops per second. Adjust vacuum strength accordingly. Finally, release the vacuum.

7 Wash silica membrane

1st wash

Add **400 µL PW1** to each well of the NucleoSpin® Plant II Binding Plate and apply vacuum of **-0.2 to -0.4 bar*** until the buffer has passed the membrane completely. Release the vacuum.

2nd wash

Add **700 µL PW2** to each well of the NucleoSpin® Plant II Binding Plate and apply vacuum of **-0.2 to -0.4 bar*** until the buffer has passed the membrane completely. Release the vacuum.

* Reduction of atmospheric pressure

3rd wash

Add 700 µL PW2 to each well of the NucleoSpin® Plant II Binding Plate and apply vacuum of -0.2 to -0.4 bar* until the buffer has passed the membrane completely. Release the vacuum.

Remove MN Wash Plate

After the final washing step close the valve, release the vacuum and remove the NucleoSpin® Plant II Binding Plate. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Optional: If necessary move the NucleoSpin® Plant II Binding plate to a clean paper sheet (included with the MN Wash Plate) to remove residual wash buffer.

Reassemble the vacuum manifold and dry the membrane by applying maximum vacuum (e.g., -0.6 bar*) for 10 minutes.

8 Elute DNA

Insert spacers 'MICROTUBE RACK' into the vacuum manifold base. Place the Rack of Tube Strips into the manifold base. Close the manifold and insert the NucleoSpin® Plant II Binding Plate into the manifold top. Dispense **100 µL preheated Buffer PE (70 °C)** to each well of the NucleoSpin® Plant II Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for 2 min. Apply vacuum of **-0.4 bar*** until the elution buffer has passed the membrane completely.

For optimal yield it is recommended to repeat this step once (incubation of Buffer PE on the membrane not required)

Yields will be 10–20 % higher when eluting with 2 x 100 µL Buffer PE depending on the total amount of DNA. However, the concentration of DNA will be much lower than with 100 µL.

Note: Elution can be done with TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH ≤ 8.0.

* Reduction of atmospheric pressure

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
DNA yield is low	*Homogenization of plant material was not sufficient* • For most species we recommend grinding with steel beads. Homogenization should be done thoroughly until the plant material is ground to a fine powder. In most cases this can be achieved by vigorous shaking for 3 x 60 s with occasional freezing in liquid nitrogen. • This problem can also be avoided by lyophilizing the material. This way, it will be easier to grind the material. *Extraction of DNA from plant material during lysis was not sufficient* • To obtain higher yields of DNA, the incubation time in lysis buffer can be prolonged (up to overnight). *Suboptimal lysis buffer was used* • Lysis efficiencies of Buffer PL1 (CTAB) and Buffer PL2 (SDS) are different and depend on the plant species. Try both buffers in a side-by side purification to find the best detergent system to lyse your plant material. *Sample contains too much RNA* • Add 10 µL of RNase A solution to the Lysis Buffer PL1 or PL2 before heat incubation. If this is not successful, add the enzyme to the cleared supernatant of step 3 and incubate for 30 min at 60 °C. *Sub-optimal Elution* • The DNA can be either eluted in higher volumes (up to 300 µL) or by repeating the elution step up to three times. Remember that the elution buffer must be preheated to 70 °C prior to elution. • Also check the pH of the elution buffer used, which should be in a range of pH 8–8.5. To ensure correct pH, use supplied elution Buffer PE.
DNA is degraded	*Sample was contaminated with DNase* • Check bench, pipettes and storage of sample in order to avoid DNase contamination.

Problem	Possible cause and suggestions
DNA purity is low	*Sample contains DNA-degrading contaminants (e.g., phenolic compounds, secondary metabolites)* • Repeat washing step with Buffer PW1. *Elution buffer contains EDTA* • EDTA can disturb subsequent reactions. Use of water or supplied Elution Buffer PE is highly recommended.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 Plant II	740663.2 740663.4 740663.24	1 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoSpin® 96 Plant II Core Kit	740468.4	4 x 96 preps
NucleoSpin® 8 Plant II	740669 740669.5	12 x 8 preps 60 x 8 preps
NucleoSpin® 8 Plant II Core Kit	740467.4	48 x 8 preps
MN Bead Plate Type D	740853.1 740853.4 740853.24	pack of 1 pack of 4 pack of 24
MN Bead Tube Type G	740817.50	50 pieces
Buffer PL1	740918	125 mL
Buffer Set PL2 / PL3 (100 mL Buffer PL2 + 25 mL Buffer PL3)	740919	1 set
Buffer PC	740937	125 mL
Buffer PW1	740938	125 mL
Buffer PW2 Concentrate (for 250 mL Buffer PW2)	740939	50 mL
RNase A (lyophilized)	740505 740505.50	100 mg 50 mg
Proteinase K	740506	100 mg
MN Square-well Block	740476 740476.24	4 24
MN Wash Plate	740479 740479.24	4 24
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
Gas-permeable Foil	740675	50
Self adhering Foil	740676	50

Product	REF	Pack of
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
MN Positive Pressure Frame	740474	1

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-NAGELS employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

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

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

Last updated: 08/2022, Rev. 04

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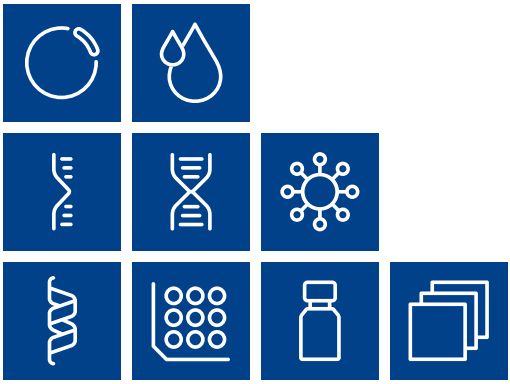
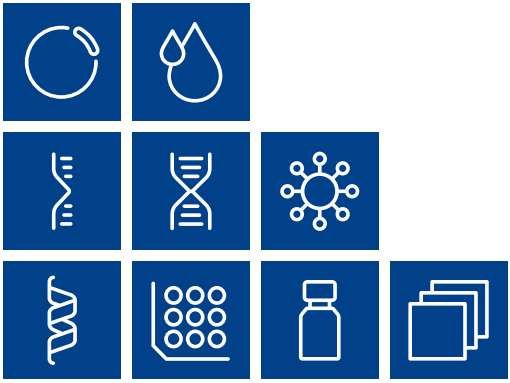
support@mn-net.com



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



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