Genomic DNA from Plant

User Manual

NucleoSpin® 96 Plant II

May 2008/ Rev.02

MACHEREY-NAGEL

Table of contents

1 Components

1.1 Kit contents

	NucleoSpin® 96 Plant II		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**
Cat. No.	**740663.2**	**740663.4**	**740663.24**[1]
Lysis Buffer PL1	120 ml	240 ml	6 x 240 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	110 ml	2 x 110 ml	6 x (2 x 110 ml)
Wash Buffer PW1	100 ml	2 x 100 ml	6 x (2 x 100 ml)
Wash Buffer PW2 (Concentrate)[2]	80 ml	150 ml	6 x 150 ml
Elution Buffer PE[3]	50 ml	100 ml	6 x 100 ml
RNase A (lyophilized)[2]	30 mg	2 x 30 mg	6 x (2 x 30 mg)
NucleoSpin® Plant II Binding Plates (dark green rings)	2	4	6 x 4
MN Wash Plates	2	4	6 x 4
Tube Strips with Cap Strips[4] (for lysis and elution)	4	8	6 x 8
MN Square-well Blocks	6	12	6 x 12
Gas-permeable Foil	10	20	6 x 20
Cap Strips	24	48	6 x 48
User Manual	1	1	1

[1] The kit for 24x96 preparations Cat. No. 740663.24 consists of 6x Cat. No. 740663.4.
[2] For preparation of working solutions see section 3.
[3] Composition of Elution Buffer PE: 5 mM Tris/HCl, pH 8.5
[4] Set of 1 rack with 12 8-well Tube Strips and 12 Cap Strips

1.2 Required hardware

Centrifugation

For centrifugation a microtiterplate centrifuge which is able to accommodate the NucleoSpin® Plant II Binding Plate stacked on a MN Square-well Block or rack with Tube Strips and reaches accelerations of 5,600 – 6,000 x *g* is required (bucket height: 85 mm), e.g. Hermle Z 513/Z 513 K, Jouan KR4i, Heraeus Kendro Multifuge 3/3-R, Beckman Coulter Allegra25R, Hettich Rotanta 460 series, Sigma 4-15/4K15/6-15/6K15.

Vacuum processing

For manual processing under vacuum a NucleoVac 96 Vacuum Manifold (Cat. No. 740681) is required.

2 Product description

2.1 The basic principle

The **NucleoSpin 96 Plant II** kit is designed for the isolation of genomic DNA from a variety of 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 lysis of plant material with CTAB lysis buffer (PL1) specially developed for plants. Alternatively, a SDS based lysis buffer (PL2) is provided. 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 (PW1, PW2), DNA can be eluted in low salt buffer (PE) or water and is ready-to-use for subsequent reactions.

2.2 Kit specifications

- **NucleoSpin® 96 Plant II** is designed for the isolation of genomic DNA from plant tissue.

- **NucleoSpin® 96 Plant II** allows parallel purification of multiples of 96 samples each with up to 100 mg sample per well.

- 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 $A_{260/280}$ ratio between 1.80 and 1.90 and a typical concentration of 100-200 ng/µl. The amount of DNA that can be expected per mg of sample depends on the size and ploidy of the genome. E.g., 100 mg fresh wheat with a hexaploid genome ($1.7x10^{10}$ bp) contain 30 µg DNA, whereas the same amount of Arabidopsis with a smaller diploid genome ($1.9x10^{8}$ bp) yields 3 µg DNA only.

- The eluted DNA is ready-to-use in subsequent reactions like PCR, restriction analysis etc.

Kit specifications at a glance	
Parameters	**NucleoSpin® 96 Plant II**
Sample size	up to 100 mg
Typical DNA yield	1-30 µg
Elution volume	100 µl
DNA Binding capacity	30 µg
Processing time for 2 plates	60 min (after lysis)

2.3 Storage and homogenization of samples

We recommend using young plant samples and if possible to keep plants for about 12 h in the dark before collecting samples 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. When using dried samples reduce the amount of starting material by the factor 5, e.g. use 20 mg dried plant leaves instead of 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 (contact Saaten-Union Resistenzlabor GmbH, D-33818 Leopoldshöhe), Tissue Striker (www.KisanBiotech.com) or Geno/Grinder 2000 (www.spexcsp.com or for Germany www.c3-analysentechnik.de)

- 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, contact 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 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.

- 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. Close the individual tubes with Cap Strips. Freeze the sample in liquid nitrogen and insert the rack with Tube Strips in a suitable homogenization tool (e.g. mixer mill). For disruption shake the samples for 60-90 sec at 30 Hz or until a homogenous plant powder has formed. If necessary repeat shaking once. Fresh plant material can also be homogenized with lysis buffer. Homogenization of fresh plant material with lysis buffer may cause shearing of DNA. For frozen plant material thawing should be avoided during the homogenization. Sample 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 may result in higher yield but also may cause shearing of DNA.

2.4 Elution procedures

It is possible to adapt elution method and volume of elution buffer to the subsequent 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 e.g. 2 x 100 µl. Finally, combine eluates and measure yield.

- **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.

- **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.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and/or multi-use storage at 4°C or ambient temperature by inhibition of 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 elution with the supplied elution buffer and storage, 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 detection sensitivity of trace amount of DNA species might be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at 4°C or room temperature due to shearing of DNA or adsorption to surfaces.

3 Storage conditions and preparation of working solutions

Attention:

Buffers PL1, PL2, PC and PW1 contain guanidinium hydrochloride, and/or detergents like CTAB or SDS! Wear gloves and goggles!

- Store RNase A at 4°C on arrival. All other kit components are stable at **room temperature**. (Storage at 4°C may cause precipitation of salts in different buffers.)

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 given volume of ethanol (96-100%) to **Buffer PW2 Concentrate** before first use. Store Buffer PW2 at room temperature (20-25°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		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**
Cat. No.	**740663.2**	**740663.4**	**740663.24**
Wash Buffer PW2 (Concentrate)	80 ml add 320 ml ethanol	150 ml add 600 ml ethanol	6 x 150 ml add 600 ml ethanol to each bottle
RNase A (lyophilized)	1 x 30 mg add 2.5 ml water	2 x 30 mg add 2.5 ml water to each vial	6 x (2 x 30 mg) add 2.5 ml water to each vial

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® 96 Plant II kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
PC	Guanidine hydrochloride + ethanol < 40 %	Xn	Flammable. Harmful if swallowed. Irritating to eyes and skin	R 10-22-36/38	S 7-16
PW1	Guanidine hydrochloride + isopropanol < 25%	 Xn	Flammable. Harmful if swallowed. Irritating to eyes and skin	R 10-22-36/38	S 7-16-25
RNase A	RNase A, lyophilized	 Xn*	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24

Risk Phrases

R 10 Flammable

R 22 Harmful if swallowed

R 36/38 Irritating to eyes and skin

R 42/43 May cause sensitization by inhalation and skin contact

Safety Phrases

S 7 Keep container tightly closed

S 16 Keep away from sources of ignition – No Smoking!

S 22 Do not breathe dust

S 24 Avoid contact with the skin

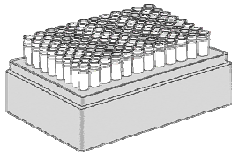
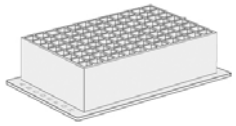
S 25 Avoid contact with the eyes

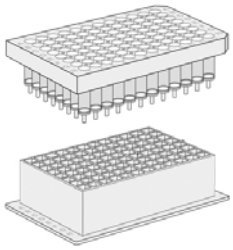
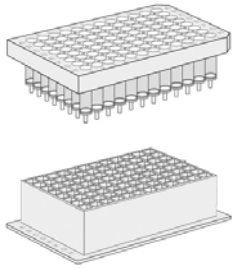
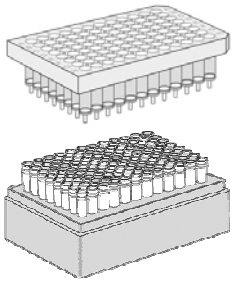
* Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

5 General procedure

NucleoSpin® 96 Plant II kit, centrifuge processing

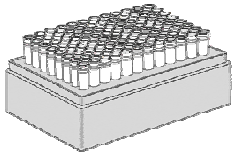
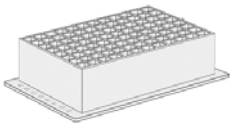
For detailed information on each step see section 5.1.

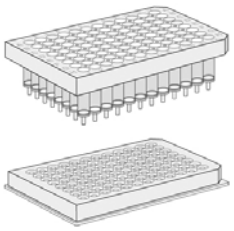
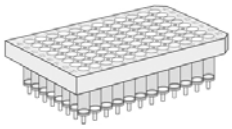
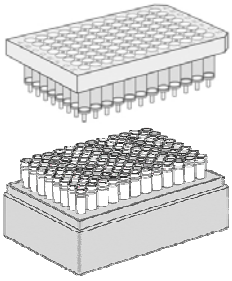
1	Homogenize samples	**100 mg wet or 20 mg lyophilized plant tissue**	
2a	Cell lysis using Buffer PL1	**500 µl PL1 (10 µl RNase A)** **mix** **65 °C, 30 min** **proceed with step 3**	
2b	Cell lysis using Buffer PL2 and PL3	**400 µl PL2 (10 µl RNase A)** **mix** **65 °C, 30 min** **100 µl PL3** **mix and incubate on ice for 5 min** **proceed with step 3**	 Rack with Tube Strips
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**	 MN Square-well Block
5	Transfer lysate to NucleoSpin® Plant II Binding Plate		

6	Bind DNA to silica membrane of the NucleoSpin® Plant II Binding Plate	**5,000 – 6,000 x *g* (2 min)**	 NucleoSpin® Plant II Binding Plate and MN Square-well Block
7	Wash silica membrane	**400 µl PW1** **5,000 – 6,000 x *g*; 2 min** **700 µl PW2** **5,000 – 6,000 x *g*; 2 min** **700 µl PW2** **5,000 – 6,000 x *g*; 10 min**	
8	Elute highly pure total DNA	**100 µl PE (incubate 2 min)** **5,000 – 6,000 x *g* (2 min)** **repeat once**	 NucleoSpin® Plant II Binding Plate and rack with Tube Strips

NucleoSpin® 96 Plant II kit, vacuum processing

For detailed information on each step see section 5.2.

1	Homogenize samples	**100 mg wet or 20 mg lyophilized plant tissue**	
2a	Cell lysis using Buffer PL1	**500 µl PL1** **(10 µl RNase A)** **mix** **65 °C, 30 min** **proceed with step 3**	 Rack with Tube Strips
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	**5,000 – 6,000 x *g*** **(20 min)**	
4	Adjust binding conditions	**mix 450 µl PC with 400 µl cleared lysate**	 MN Square-well Block
5	Transfer lysate to NucleoSpin® Plant II Binding Plate		

6	Bind DNA to silica membrane of the NucleoSpin® Plant II Binding Plate	**- 200 to - 400 mbar* (2 min)**	
7	Wash and dry silica membrane	**400 µl PW1** **700 µl PW2** **700 µl PW2** **- 400 mbar*** **(1 min each step)**	NucleoSpin® Plant II Binding Plate and MN Wash Plate
		remove MN Wash Plate	
		dry silica membrane (10 min, maximum vacuum)	NucleoSpin® Plant II Binding Plate
8	Elute highly pure total DNA	**100 µl PE (incubate 2 min)** **- 400 mbar*** **(2 min)**	
			NucleoSpin® Plant II Binding Plate and rack with Tube Strips

* Reduction of atmospheric pressure

5.1 NucleoSpin® 96 Plant II protocol – purification of genomic DNA from plants, centrifuge processing

Before starting with the preparation, set incubator or oven to 65°C. Equilibrate Buffer PE to 70°C. Prepare Buffer PW2, and RNase A solution (see section 3).

1 Homogenize samples

Fill up to **100 mg wet plant tissue** (or up to **20 mg dried, e.g. 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 5 min and remove Cap Strips.

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

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 sec**. **Spin briefly for 30 sec 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 sec**. **Spin** briefly for **30 sec 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.

Add **100 µl Buffer PL3**, 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

Pre-dispense **450 µl Binding Buffer PC** to each well of an 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 an 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.

Optional: Seal plate with a Gas-permeable Foil. Centrifuge again at 5,600 – 6,000 × 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.

*Optional: Seal plate with a Gas-permeable Foil. Centrifuge again at **5,600 – 6,000 × g** for **2 min.***

3rd wash

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

*Optional: Seal plate with a Gas-permeable Foil. Centrifuge again at **5,600 – 6,000 × g** for **10 min** for complete removal of ethanolic Buffer PW2.*

8 Elute highly pure DNA

Place NucleoSpin® Plant II Binding Plate on the rack with Tube Strips. Dispense **100 µl pre-warmed 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.

Optional: Incubate plate for 2 min at 70°C before centrifugation. Centrifuge at 5,600 – 6,000 × g for 2 min. Remove the NucleoSpin® Plant II Binding Plate from the rack with 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. The concentration of DNA, however, will be much lower than with 100 µl then.

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 protocol – purification of genomic DNA from plants, vacuum processing

Before starting with the preparation, set incubator or oven to 65°C. Equilibrate Buffer PE to 70°C. Prepare Buffer PW2, and RNase A solution (see section 3).

1 Homogenize samples

Fill up to **100 mg wet plant tissue** (or up to **20 mg dried, e.g. lyophilized, plant tissue**) into each tube of the rack with 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 5 min and remove Cap Strips.

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

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 sec**. **Spin** briefly for **30 sec 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 Buffer 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 sec**. **Spin** briefly for **30 sec 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.

Add **100 µl Buffer PL3**, 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

Pre-dispense **450 µl Binding Buffer PC** to each well of an 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 **-200 to -400 mbar*** to allow samples to pass through the membrane. Flow-through rate should be about 1-2 drops per second. Adjust vacuum strength accordingly.

7 Wash silica membrane

1st wash

Add **400 µl PW1** to each well of the NucleoSpin® Plant II Binding Plate and apply vacuum of **-200 to -400 mbar*** until the buffer has passed the membrane completely. Release vacuum.

2nd wash

Add **700 µl PW2** to each well of the NucleoSpin® Plant II Binding Plate and apply vacuum of **-200 to -400 mbar*** until the buffer has passed the membrane completely. Release vacuum.

3rd wash

Add **700 µl PW2** to each well of the NucleoSpin® Plant II Binding Plate and apply vacuum of **-200 to -400 mbar*** until the buffer has passed the membrane completely. Release vacuum.

Remove MN Wash Plate and waste tray.

Reassemble the vacuum manifold and dry the membrane by applying maximum vacuum (**-600 mbar***) for 15 minutes.

* Reduction of atmospheric pressure

8 Elute highly pure DNA

Insert spacers “Microtube Rack” into the vacuum manifold base. Place the rack with Tube Strips into the manifold base. Close the manifold and insert the NucleoSpin® Plant II Binding Plate into the manifold top. Dispense **100 µl pre-warmed 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 **-400 mbar*** 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. The concentration of DNA, however, will be much lower than with 100 µl then.

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 sec with occasional freezing in liquid nitrogen. • This problem can also be circumvented by lyophilizing the material. In this case grinding of the material becomes easier. *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	Cat. No.	Pack of
NucleoSpin® 96 Plant II	740663.2	2 x 96 preps
NucleoSpin® 96 Plant II	740663.4	4 x 96 preps
NucleoSpin® 96 Plant II	740663.24	24 x 96 preps
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	740505	100 mg
RNase A	740505.50	50 mg
Proteinase K	740506	100 mg
MN Square-well Block	740 678	20
Tube Strips*	740 637	5 racks
Cap Strips	740 638	30

* Set of 5 racks with 12 8-well Tube Strips

Product	Cat. No.	Pack of
Gas-permeable Foil	740675	50 sheets
MN Wash Plate	740 479	4
MN Wash Plate	740 674	20
NucleoVac 96 Vacuum Manifold	740 681	1
Vacuum Regulator	740 641	1

6.3 Product use restriction / warranty

NucleoSpin® 96 Plant II kit components were developed, designed, distributed and sold **for RESEARCH PURPOSES ONLY**. They are suitable ***for IN – VITRO USES only***. Furthermore is no claim or representation intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather in the responsibility of the user to verify the use of the **NucleoSpin® 96 Plant II** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL guarantees to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

MACHEREY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; against defects in products or components not manufactured by MACHEREY-NAGEL, or against damages resulting from such non-MACHEREY-NAGEL components or products.

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Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

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