Genomic DNA from Plant

User manual

NucleoSpin® 8 Plant

NucleoSpin® 96 Plant

April 2004/ Rev. 01

MACHEREY-NAGEL

Table of contents

1 Kit contents

	NucleoSpin® 8 Plant[1]	
	12 x 8 preps	**60 x 8 preps**
Cat.No.	**740662**	**740662.5**
Buffer C1	60 ml	300 ml
Buffer C2	50 ml	200 ml
Buffer C3	12.5 ml	50 ml
Buffer CW	75 ml	2 x 150 ml
Buffer C5[2] (concentrate)	50 ml	2 x 100 ml
Buffer CE	25 ml	125 ml
NucleoSpin® Plant Binding Strips (green)	12	60
RNase A	30 mg	2 x 30 mg
Rack with MN Tube Strips (Lysis) with Cap Strips (blue rack)	1	5
Rack with MN Tube Strips (Elution) with Cap Strips (white rack)	1	5
MN Wash Plate	2	10
Gas-permeable Foils	6	18
Round-well Block	1	5
Protocol	1	1

[1] The use of NucleoSpin 8 Plant requires a Starter Set containing additional hardware (see section 2.3).
[2] For preparation of working solutions and storage conditions see section 3.

1 Kit contents *continued*

	NucleoSpin® 96 Plant		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**[3]
Cat.No.	**740661.2**	**740661.4**	**740661.24**
Buffer C1	100 ml	300 ml	6 x 300 ml
Buffer C2	80 ml	200 ml	6 x 200 ml
Buffer C3	20 ml	50 ml	6 x 50 ml
Buffer CW	125 ml	250 ml	6 x 250 ml
Buffer C5 (concentrate)[4]	80 ml	2 x 80 ml	12 x 80 ml
Buffer CE	50 ml	75 ml	6 x 75 ml
NucleoSpin® Plant Binding Plate (green)	2	4	24
RNase A	30 mg	2 x 30 mg	12 x 30 mg
Rack with MN Tube Strips (Lysis) with Cap Strips	2	4	6 x 4
Rack with MN Tube Strips (Elution) with Cap Strips	2	4	6 x 4
MN Wash Plate	2	4	6 x 4
Round-well Block with Cap Strips	2	4	6 x 4
Gas-permeable Foils	6	12	6 x 12
MN Square-well Block	2	2	6 x 2
Protocol	1	1	6 x 1

[3] The kit for 24x96 preparations Cat. No. 740 661.24 consits of 6 x Cat. No. 740 661.4
[4] For preparation of working solutions and storage conditions see section 3.

2 Product description

2.1 The basic principle

With the NucleoSpin® 8/96 Plant method, genomic DNA is prepared from plant tissues. 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 buffers specially developed for plants. Lysis mixtures should be cleared by centrifugation or filtration in order to remove polysaccharides, contaminations and residual cellular debris. The clear supernatant is mixed with binding buffer and ethanol to create conditions for optimal binding to the silica membrane in the NucleoSpin® Plant Binding Module. After washing with two different buffers, DNA can be eluted in low salt buffer or water and is ready-to-use for subsequent reactions.

Plants are very heterogeneous and contain many different metabolites like polyphenolics, polysaccharides or acidic components. To address this problem different protocols are given for the optimal processing of various samples.

2.2 Kit specifications

- **NucleoSpin® 8/96 Plant** system is designed for the isolation of genomic DNA from plant tissue and other biological samples like soil.
- **NucleoSpin® 8/96 Plant** kits allow parallel purification of multiples of 8/96 samples each with ≤ 50 mg material.
- Depending on the individual sample, **NucleoSpin® 8/96 Plant** yield in the range of 5-20 µg DNA with an $A_{260/280}$ ratio between 1.80 and 1.90. The membrane capacity of each well is about 25 µg.
- The eluted DNA is ready-to-use in subsequent reactions like PCR, restriction analysis, etc.
- **NucleoSpin® 8/96 Plant** can be processed by centrifugation or completely under vacuum. Processing under vacuum allows 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 2.6 and contact your local distributor or MN directly.
- Any unused wells of the **NucleoSpin® 8/96 Plant Binding Module** should be covered with a Rubber Pad or Self-adhering PE Foil (see ordering information) in order to guarantee a proper vacuum and to protect the unused wells from being contaminated.

Kit specifications at a glance	
	NucleoSpin® 8/96 Plant
Sample size	up to 20 – 100 mg
Average yield	5 – 30 µg
Elution volume	100 – 200 µl
Binding capacity	25 µg
Time / 12 strips or one plate	less than 2 h

2.3 Required hardware

NucleoSpin® 8 Plant:

The NucleoSpin® 8 Plant kit can be used **manually** with the NucleoVac 96 vacuum manifold (Ca. No. 740681) or similar suitable vacuum manifolds (see section 2.4) by using the Starter Set A containing Column Holders A and Dummy Strips (see ordering information).

For **automation** on laboratory platforms with standard 96-well plate vacuum chambers the use of the Starter Set A is also required.

For **centrifugation** a microtiterplate centrifuge which is able to accommodate the NucleoSpin® Plant Binding Strips/Plate stacked on a round or square-well block and reaches accelerations of 5,600 – 6,000 x g is required (bucket height: 85 mm), e.g. Hermle/MACHEREY-NAGEL: NucleoSwing Z513, Qiagen/Sigma 4-15c, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, Highplate™, Beckman Coulter, Allegra R). Furthermore, Starter Set C (see ordering information), containing Column Holders C, Dummy Strips, MN Square-well Blocks, Tube Strips is required. For detailed information refer to the Starter Set C manual.

NucleoSpin® 96 Plant:

The **NucleoSpin® 96 Plant** kit can be used with either the NucleoVac 96 (Cat. No. 740681) or other common vacuum manifolds, e.g. the Qiagen QIAvac 96.

2.4 Suitability for other common vacuum manifolds

The **NucleoSpin® 8/96 Plant** kits can be used with other common vacuum manifolds. For further details see list below.

Vacuum manifold	Suitability	Additional equipment
Qiagen/QIAvac 96*	yes	MN Frame (see ordering information)

*In general the QIAvac 96 is suitable for the use with the NucleoSpin® Plant Binding Module. Nevertheless, it is recommended to use the MN Frame to adjust the proper height of the MN Wash Plate and Elution Plate, U-Bottom in order to ensure best performance.

2.5 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 (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 Binding Strip/Plate** for 3 min at 60-70°C. Afterwards add another volume elution buffer (e.g. 50 µl) and incubate further 2 min at room temperature before centrifugation for optimal recovery of bound nucleic acids with standard volumes of elution buffer.

Recovery of gDNA from the membrane depends on the elution volume. Elution volumes of 50 – 200 µl are possible, with an optimum of 100 – 125 µl dispensed volume. The purity is not effected by the elution volume. See table for correlation between dispensed elution buffer volume and typical recoveries following the standard protocol.

Dispensed elution buffer	75 µl	100 µl	125 µl	150 µl	175 µl
Recovered elution buffer containing plasmid DNA	30±5µl	55±5 µl	80±5 µl	105±5 µl	130±5 µl

2.6 Automation

NucleoSpin® 8/96 Plant can be fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 8/96 Plant** on a certain workstation please contact MN. Full processing under vacuum enables complete automation without the need for centrifugation steps for drying of the 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® 8/96 Plant Binding Module**.

Drying of the **NucleoSpin® 8/96 Plant Binding Module** under vacuum is sufficient because the bottom of the plate/strip is protected from spraying wash buffer during the washing steps by the MN Wash Plate. So, if possible the MN Wash Plate should be integrated into the automated procedure. The MN Frame (Cat. No. 740 680) can be used to position the 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 manifold is recommended after each run to prevent forming of gDNA-containing aerosols.

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

2.7 Storage and homogenization of samples

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

Plant samples can be stored frozen, under ethanol or lyophilized. In many cases lyophilized, dried material can be easier processed and gives higher yield. If 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: 7 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.

3 Storage conditions and preparation of working solutions

Attention:

Buffers C1-3 and CW contain guanidinium hydrochloride, CTAB and detergents! 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® 8/96 Plant** protocol prepare the following:

- **Buffer C4:** Transfer the total contents of buffer C3 to buffer C2 and mix well. The resulting buffer C4 is stable for 4 months at room temperature (to be stored in the dark). For a better disolving of both components a 5 min incubation at 45°C is recommended.

- If the kit will only be used occasionally, it is also possible to mix one volume of buffer C3 with four volumes of buffer C2, e.g. 100 µl buffer C3 and 400 µl buffer C2. Mix by pipetting up and down

- **Buffer C5:** Add the given volume of ethanol (indicated on the bottle) to **buffer C5** before use.

- **RNase A:** Add the given volume of water (indicated on the tube) to **RNase A.** Store at 4°C.

	NucleoSpin® 8 Plant	
	12 x 8 preps	**60 x 8 preps**
Cat.No.	**740662**	**740662.5**
Buffer C5 concentrate	50 ml add 200 ml ethanol	100 ml add 400 ml ethanol
RNase A	30 mg dissolve in 2.5 ml H_2O	2 x 30 mg dissolve in 2.5 ml H_2O each

	NucleoSpin® 96 Plant	
	2 x 96 preps	**4 x 96 preps**
Cat.No.	**740661.2**	**740661.4**
Buffer C5 concentrate	80 ml add 320 ml ethanol	80 ml add 320 ml ethanol
RNase A	30 mg dissolve in 2.5 ml H_2O	2 x 30 mg dissolve in 2.5 ml H_2O each

The kit for 24 x 96 preparations (Cat.No. 740 661.24) consists of 6 x Cat.No. 740 665.4

4 Safety Instructions – Risk and Safety Phrases

The following components of the NucleoSpin® 8/96 Plant kits contain hazardous contents.

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

Buffer/ Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
C2	**guanidine hydrochloride**	Xn*	**Harmful if swallowed. Irritating to eyes and skin**	**R 22-36/38**	**S 22**
CW	**guanidine hydrochloride**	Xn*	**Harmful if swallowed. Irritating to eyes and skin**	**R 22-36/38**	**S 22**
RNase A	**RNase A, lyophilized**	Xn*	**May cause sensitization by inhalation and skin**	**R 42/43**	**S 7-16-22**

Risk Phrases

R 22 Harmful if swallowed

R 36/38 Irritating to eyes and skin

R 42/43 May cause sensitisation 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

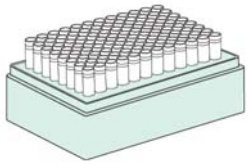
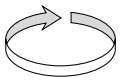
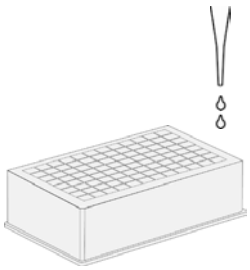
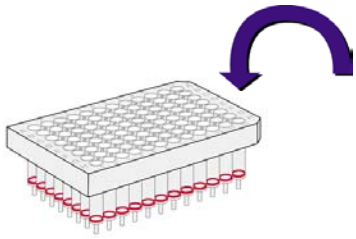
* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

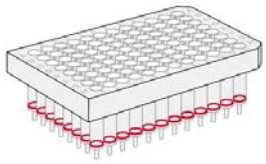
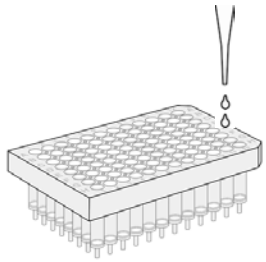
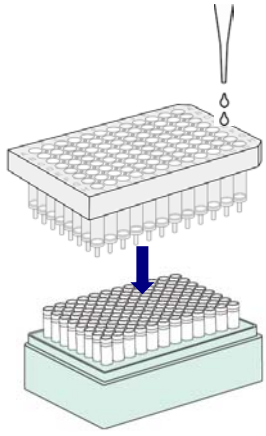
5 General Procedure NucleoSpin® 8/96 Plant

NucleoSpin® 8/96 Plant uses a combination of CTAB extraction and subsequent binding to a special silica membrane. The detergent CTAB (cetyl trimethyl ammonium bromide) is able to remove polysaccharides and other contaminants, while DNA remains in solution. The resulting clear lysate is mixed with buffer C4 in order to adjust binding conditions for the **NucleoSpin® Plant membrane**. After loading this mixture onto the plate, contaminants are washed away with washing buffers CW and C5. The genomic DNA can finally be eluted with the low salt elution buffer CE.

The following plant species have been tested with the standard protocol:

Arabidopsis thaliana, Brassica napus (rape), Helianthus spp. (sunflower), Hordeum vulgare (barley), Nicotiana tabacum (tobacco), Solanum tuberosum (potato), Vicia narbonensis (narbonne vetch), Zea mays (maize), Poa spp. (grass), Pisum arvense (pea), Allium cepa (onion), Allium sativum (garlic), Cerasus avium (sweet cherry), Saccharum officinarum (sugar cane).

1	Homogenize and lyse plant samples	**Homogenization,** **400 µl buffer C1** **mix** **56°C, 30 min**	
2	Clear lysate	**5,600 × *g*, 20 min**	
3	Adjust DNA binding conditions	**300 µl clear lysate,** **add 300 µl buffer C4 and 200 µl ethanol,** **mix**	
4	Load samples	**Transfer samples to NucleoSpin® Plant Binding Module (green)**	

5	Bind DNA to silica membrane	**5,600 × *g*, 10 min** **or** **ca. –0.2 bar* (5 min)**	
6	Wash silica membrane	**500 µl CW** **5,600 × *g*, 2 min** **or** **ca. –0.2 bar (5 min)** **900 µl C5** **5,600 × *g*, 5 min** **or** **ca. –0.2 bar (5 min)** **vacuum processing only:** **repeat C5 wash step once**	
	Remove MN Wash Plate (vacuum processing only)		
	Dry silica membrane	**5,600 x g, 15 min** **or** **37°C, 20 min** **or** **ca. –0.6 bar, 10 min**	
7	Elution	**100 µl CE, 70°C** **5,600 × *g*, 2 min** **or** **ca. –0.6 bar, 2 min** **optional** **repeat elution step once**	

* reduction of atmospheric pressure

5.1 NucleoSpin® 8 Plant Protocol – purification of DNA under centrifugation

Before starting with preparation, prepare buffers C4 and C5 and RNase A solution (see section 3 for details). Equilibrate buffer CE to 70°C.

For each preparation collect up to 50 mg of plant tissue into an appropriate lysis vessel, e.g. Rack with MN Tube Strips (Lysis).

1 Homogenize and lyse plant samples

Homogenize about 50 mg plant tissue by e.g. grinding in liquid nitrogen. Transfer homogenized samples into lysis vessel and add 400 µl C1. Close the lysis vessel and mix by vigorous shaking for 15-30 sec. Spin briefly for 30 sec at 1,500 x *g* to collect any sample at the bottom of the vessel. Incubate at 56°C for 30 min.

Depending on plant sample and available methods, buffer C1 may be added to the plant material in lysis vessel before homogenization by the appropriate mechanical method.

We recommend to add 10 µl RNase A to the C1 lysis solution.

2 Clear lysate

Centrifuge the samples for 10 min at full speed (11,000 x g).

Insert the desired number of NucleoSpin® Plant Binding Strips in the Column Holder C and place it on an MN Square-well Block (supplied with Starter Set C) for collection of flow through. If using more than one block label the column holders for later identification.

Always use two column holders with identical numbers of 8-well strips for centrifugation. Doing so there is no need for balancing. We recommend positioning of the 8-well strips around the center of the column holder.

3 Adjust binding conditions

Transfer 300 µl clear supernatant to a Round-well Block. Add 300 µl buffer C4 and 200 µl ethanol, Close the individual wells with Cap Strips. Mix by vigorous vortexing for 15-30 sec (or by pipetting up and down). Spin briefly for 30 sec at 1,500 x *g* to collect any sample on the bottom of the vial.

Buffer C4 and ethanol can be premixed.

4 Loading

Transfer samples from the previous step into the wells of the NucleoSpin® Plant Binding Strips. Do not moisten the rims of the individual wells while dispensing the samples. After transfer seal the openings of the strips with Gas-permeable Foil.

This foil can be cut into appropriate pieces due to the number of NucleoSpin® Plant Binding Strips which are to be covered.

Note: When not using air permeable foil pierce foil to achieve air permeability.

5 Bind DNA to silica membrane

Place the Column Holder C holding the NucleoSpin® Plant Binding Strips onto a MN Square-well Block (supplied with the Starter Set C) and place it into the rotor buckets. Centrifuge at 5,600 – 6,000 × *g* for 10 min.

Typically, the lysates will have passed through the silica membrane within a few minutes. The centrifugation process can be extended to 20 min, if the lysates have not passed completely. The volume of each well of the NucleoSpin® Plant Binding Strip is ~ 1 ml. Higher volumes, resulting from steps 1-3, have to be loaded successively until the complete lysis mixture has been applied.

6 Wash silica membrane

1st wash

Remove the Gas-permeable Foil and add 500 µl CW to each well of the NucleoSpin® Plant Binding Strips. Seal the strips with a new Gas-permeable Foil and centrifuge again at 5,600 – 6,000 × *g* for 2 min. Discard waste collected in the MN Square-well Block after this wash step.

Depending on the sensitivity of subsequent reactions this washing step is helpful in order to remove contaminants and potential PCR inhibitors.

2nd wash

Remove the Gas-permeable Foil and add 900 µl C5 to each well of the NucleoSpin® Plant Binding Strips. Centrifuge for 5-15 min at full speed (5,600 - 6,000 x g) in order to remove buffer C5.

For critical ethanol-sensitive applications it is recommedned to prolong the centrifugation time up to 15 min or incubate at higher temperature. Remove the adhesive foil and place the column holder with the NucleoSpin® Plant Binding Strips into an incubator for 20 min at 37°C to evaporate residual ethanol.

Removal of ethanol by evaporation at 37°C is more effective than additional, prolonged centrifugation (15 min, 6,000 x g).

7 Elute highly pure DNA

Place column holder with NucleoSpin® Plant Binding Strips on an opened rack with MN Tube Strips (elution). Dispense 100 µl pre-warmed buffer CE (70°C) to each well of the NucleoSpin® Plant Binding Strips. Dispense the buffer directly onto the membrane. Incubate at room temperature for 2-3 min. Centrifuge at 5,600 – 6,000 × *g* for 2 min. Remove the Column Holder C from the Tube Strips.

Yields will be 10 – 20% higher when eluting in 200 µl buffer CE depending on the total amount of DNA. The concentration of DNA, however, will be much lower than with 100 µl. Elution can also be done in TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH ≤ 8.0.

Clean the MN Square-well Blocks with detergent and hot water and incubate for 1 - 5 min in 0.4 M HCl. Rinse with water again and autoclave before next use.

5.2 NucleoSpin® 96 Plant Protocol – purification of DNA under centrifugation

Before starting with preparation, prepare buffers C4 and C5 and RNase A solution (see section 3 for details). Equilibrate buffer CE to 70°C.

1 Homogenize and lyse sample material

Fill up to 50 mg plant tissue in each well of the Rack with MN Tube Strips (Lysis). Add one 3 mm diameter steel bead to each well. Close the wells with Cap Strips. Freeze samples in liquid nitrogen. Disrupt cells by vigourous shaking using Genogrinder or mixer mill. Spin at 5,600 x g for 2 min and remove Cap Strips. Add 400 µl C1 and close wells again using Cap Strips. 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 the closed MN Tube Strips at 56°C for 30 min.

Depending on plant sample and available methods, buffer C1 may be added to the plant material before homogenization by the appropriate mechanical method.

We recommend to add 10 µl RNase A to the C1 lysis solution.

2 Clear lysate

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

3 Adjust binding conditions

Transfer 300 µl clear supernatant to a Round-well Block. Add 300 µl buffer C4 and 200 µl ethanol. Close the individual wells with Cap Strips. Mix by vigorous shaking for 15-30 sec (or pipette up and down). Spin briefly for 30 sec at 1,500 x *g* to collect any sample from cap strips.

Buffer C4 and ethanol can be premixed.

4 Loading

Place the NucleoSpin® Plant Binding Plate on an MN Square-well Block. Remove the first Cap Strip and transfer samples from the Round-well Block into the wells of the NucleoSpin® Plant Binding Plate. Do not moisten the rims of the individual wells while dispensing samples. After transfer seal the openings of the NucleoSpin® Plant Binding Plate with Gas-permeable Foil.

5 Bind DNA to silica membrane

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

Typically, the lysates will have passed through the silica membrane within a few minutes. The centrifugation process can be extended to 20 min, if the lysates have not passed completely.

The volume of each well of the NucleoSpin® Plant Binding Plate is ~ 1 ml. Higher volumes, resulting from steps 1-3, have to be loaded successively until the complete lysis mixture has been applied.

6 Wash silica membrane

1st wash

Remove the Gas-permeable Foil and add 500 µl CW to each well of the NucleoSpin® Plant Binding Plate. Seal the strips with a new Gas-permeable Foil and centrifuge again at 5,600 – 6,000 × *g* for 2 min. Discard waste collected in the MN Square-well Block after this wash step.

Depending on the sensitivity of subsequent reactions this washing step is helpful in order to remove contaminants and potential PCR inhibitors.

2nd wash

Remove the Gas-permeable Foil and add 900 µl C5 to each well of the NucleoSpin® Plant Binding Plate. Centrifuge for 5-15 min at full speed (5,600 - 6,000 x g) in order to remove buffer C5.

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 Binding Plates into an incubator for 20 min at 37°C to evaporate residual ethanol.

Removal of ethanol by evaporation at 37°C is more effective than additional, prolonged centrifugation (15 min, 6,000 x g).

7 Elute highly pure DNA

Place the NucleoSpin® Plant Binding Plate on an opened Rack with MN Tube Strips (elution). Dispense 100 µl pre-warmed buffer CE (70°C) to each well of the NucleoSpin® Plant Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for 2-3 min. Centrifuge at 5,600 – 6,000 × *g* for 2 min. Remove the plate from the Tube Strips.

Yields will be 10 – 15 % higher when eluting in 200 µl CE. The concentration of DNA, however, will be much lower. Elution can be done in TE buffer (at least pH 8.0) as well.

Yields will be 10 – 20% higher when eluting in 200 µl buffer CE depending on the total amount of DNA. The concentration of DNA, however, will be much lower than with 100 µl. Elution can also be done in TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH ≤ 8.0.

Clean the MN Square-well Blocks with detergent and hot water and incubate for 1 - 5 min in 0.4 M HCl. Rinse with water again and autoclave before next use.

5.3 Processing under vacuum

Although the NucleoSpin® 8/96 Plant kit was designed for processing under centrifugation, processing under vacuum is also possible.

NucleoSpin® 8 Plant:

For manual processing under vacuum the Starter Set A and the NucleoVac 96 vacuum manifold are required (see ordering information). Starter Set A contains the Column Holders A and the Dummy Strips to close unused rows.

NucleoSpin® 96 Plant:

For processing under vacuum the NucleoVac 96 vacuum manifold is required (see ordering information).

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1 Homogenize and lyse sample material

Homogenize about 50 mg plant tissue (see section 2.7). Transfer homogenized samples into the Rack with MN Tube Strips (lysis) and add 400 µl C1. Do not moisten the rim. Close the individual wells with cap strips. 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 the closed MN Tube Strips at 56°C for 30 min.

We recommend the use of an electronic 8-channel pipetting device with extra long tips capable of holding more than 650 µl solution, for the transfer of the lysate and buffers. A good choice is the Matrix Impact2 multichannel pipettor with 102-mm-long 1,250 µl tips (Matrix # 8251).

Depending on plant sample and available methods, buffer C1 may be added to the plant material in a round-well block before homogenization by the appropriate mechanical method.

We recommend to add 10 µl RNase A to the C1 lysis solution.

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2 Clear lysate

Centrifuge the samples for 20 min at a full speed (5,600 - 6,000 x g). Remove cap strips.

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3 Adjust binding conditions

Transfer 300 µl clear supernatant to a Round-well Block. Add 300 µl buffer C4 and 200 µl ethanol. Close the individual wells with cap strips. Mix by vigorous shaking for 15-30 sec (or pipette up and down). Spin briefly for 30 sec at 1,500 x *g* to collect any sample from cap strips.

Buffer C4 and ethanol can be premixed.

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4 Loading

Insert spacers labeled “MTP/Multi-96 plate” notched side up and rest the MN Wash Plate on them. Insert waste tray into vacuum manifold base. Close manifold and place NucleoSpin® Plant Binding Module on top of the manifold. Transfer samples to the wells of the NucleoSpin® Plant Binding Module.

5 Bind DNA to silica membrane

Apply vacuum of -200 to -400 mbar (reduction of atmospheric pressure) to allow sample to pass through the membrane. Flow-through rate should be about 1-2 drops per second. Adjust vacuum strength accordingly.

6 Wash silica membrane

1st wash

Add 500 µl buffer CW to each well of the NucleoSpin® Plant Binding Module and apply vacuum of –400 mbar (reduction of atmospheric pressure).

Depending on the sensitivity of subsequent reactions this washing step is helpful in order to remove contaminants and potential PCR inhibitors.

2nd wash

Add 900 µl C5 to each well of the NucleoSpin® Plant Binding Module**.** Apply vacuum of –400 mbar (reduction of atmospheric pressure) in order to remove buffer C5. .

3nd wash

Add 900 µl C5 to each well of the NucleoSpin® Plant Binding Module**.** Apply vacuum of –400 mbar (reduction of atmospheric pressure) in order to remove buffer C5.

Remove MN Wash Plate and waste tray.

Dry the membrane by applying maximum vacuum for 15 minutes.

7 Elution

Rest the Rack with MN Tube Strips (elution) on appropriate spacers ("Microtube rack") into manifold base. Close manifold and insert NucleoSpin® Plant Binding Module into manifold top. Dispense 100 µl buffer CE (preheated to 70°C) to each well of the NucleoSpin® Plant Binding Module. Pipette buffer directly onto the membrane. Incubate at room temperature for 2 - 3 min. Apply vacuum of -400 mbar (reduction of atmospheric pressure) until all the samples have passed.

For optimal yield it is recommended to repeat this step once (incubation not necessary).

Yields will be 10 – 20% higher when eluting in 200 µl buffer CE depending on the total amount of DNA. The concentration of DNA, however, will be much lower than with 100 µl. Elution can also be done in TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH ≤ 8.0.

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 (see section 2.2). Homogenization should be done thoroughly until the plant material is ground to a fine powder. In most cases this can be achieved by vortexing for 3 x 30 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 C1 can be prolonged (up to overnight). *Sample contains too much RNA* • Add 10 - 20 µl of RNase A solution to the lysis buffer C1 before heat incubation. If this is not successful, add the enzyme to the cleared supernatant of step 2 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 60°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 CE.
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 quality is low	*Sample contains DNA-degrading contaminants (e.g. phenolic compounds, secondary metabolites)* • Repeat washing step with buffer CW. *Elution buffer contains EDTA* • EDTA can disturb subsequent reactions. Use of water or supplied elution buffer CE is highly recommended.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 8 Plant kit	740662	12 x 8 preps
NucleoSpin® 8 Plant kit	740662.5	60 x 8 preps
NucleoSpin® 96 Plant kit	740661.2	2 x 96 preps
NucleoSpin® 96 Plant kit	740661.4	4 x 96 preps
NucleoSpin® 96 Plant kit	740661.24	24 x 96 preps
Lysis buffer C1	740930	100 ml
Wash buffer C5 concentrate (for 100 ml)	740931	20 ml
Wash buffer CW	740932	100 ml
RNase A	740505	100 mg
RNase A	740505.50	50 mg
MN Square-well Block	740 678	20
Square-well Block	740 670	20
Round-well Block	740 671	20
MN Tube Strips	740 637	5 racks
Cap Strips	740 638	30
MN Wash Plates	740 674	20

Product	Cat. No.	Pack of
Self-adhering PE Foil	740 676	50
MN Frame	740 680	1
Starter Set A	740 682	1
Starter Set C	740 684	1
Vacuum Regulator	740 641	1
NucleoVac 96 vacuum manifold	740 681	1

6.3 References

Vogelstein B., and D. Gillespie. 1979. Proc. Natl. Acad. Sci. USA **76:** 615-619.

6.4 Product Use Restriction / Warranty

NucleoSpin® 8/96 Plant kits components were developed, designed 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® 8/96 Plant** 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.

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