Genomic DNA from Plant

User manual

NucleoSpin® Plant

NucleoSpin® Plant L

NucleoSpin® Plant XL

November 2007/Rev. 07

MACHEREY-NAGEL

Protocol-at-a-glance (Rev. 07)

Genomic DNA Purification from Plant

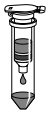
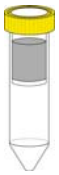
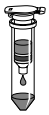
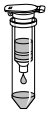
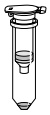
	Mini	Midi	Maxi
	NucleoSpin® Plant	NucleoSpin® Plant L	NucleoSpin® Plant XL
1 Homogenize samples	 100 mg	 250 mg	 10000 mg
2 Cell lysis	 400 µl C1 10 µl RNase A or 400 µl C0 10 µl RNase A 60°C 30 min	 C1 1.25 ml C1 20 µl RNase A 60°C, 30 min CL 1.6 ml CL 20 µl RNase A 300 µl β-ME 60°C, 30 min 0.5 ml KAc 0°C, 5 min 5 min 4,500 x *g* 	 C1 5 ml C1 80 µl RNase A 60°C, 30 min CL 8 ml CL 80 µl RNase A 1.2 ml β-ME 60°C, 30 min 2.5 ml KAc 0°C, 5 min 5 min 4,000 x *g* 
3 Filtration/ Clarification of lysate	 5 min 11,000 x *g* 	 10 min 4,500 x *g* 	 10 min 4,000 x *g* 
4 Adjust DNA binding conditions	300 µl C4 200 µl ethanol	1 vol C4 1 vol ethanol	1 vol C4 1 vol ethanol
5 Bind DNA	 1 min 11,000 x *g* 	 2 min 4,500 x *g* 	 2 min 4,000 x *g* 
6 Wash silica membrane	 1st 400 µl CW 2nd 700 µl C5 1 min 11,000 x *g* 	 1st 1 ml CQW 2nd 2 ml C5 2 min 4,500 x *g* 	 1st 4 ml CQW 2nd 10 ml C5 2 min 4,000 x *g* 
7 Wash/ Dry silica membrane	 3rd 200 µl C5 2 min 11,000 x *g* 	 10 min 4,500 x *g* 	 15 min 4,000 x *g* 
8 Elute highly pure DNA	 100 µl CE (70°C) 1 min 11,000 x *g* 	 200 µl CE (70°C) RT 5 min 3 min 4,500 x *g* 	 750 µl CE (70°C) RT 5 min 3 min 4,000 x *g* repeat 

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Table of contents

1 Kit contents

	NucleoSpin® Plant (Mini)		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740570.10**	**740570.50**	**740570.250**
Solution C	6 ml	30 ml	3 x 50 ml
Lysis Buffer C0 (Concentrate)*	150 mg	750 mg	3 x 1.2 g
Lysis Buffer C1	6 ml	30 ml	150 ml
Buffer C2	6 ml	24 ml	120 ml
Buffer C3	1.5 ml	6 ml	30 ml
Wash Buffer CW	6 ml	30 ml	125 ml
Wash Buffer C5 (Concentrate)*	6 ml	25 ml	50 ml
Elution Buffer CE**	5 ml	15 ml	30 ml
RNase A (lyophilized)*	1.5 mg	6 mg	2 x 15 mg
NucleoSpin® Filters (violet ring)	10	50	250
NucleoSpin® Plant Columns (grey ring)	10	50	250
Collection Tubes (2 ml)	20	100	500
Label for Binding Buffer C4	1	1	1
User Manual	1	1	1

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

** Composition of Elution Buffer CE: 5 mM Tris/HCl, pH 8.5

1 Kit contents *continued*

	NucleoSpin® Plant L (Midi)	NucleoSpin® Plant XL (Maxi)
	20 preps	**6 preps**
Cat. No.	**740539.20**	**740540.6**
Lysis Buffer C1	30 ml	60 ml
Lysis Buffer CL	30 ml	60 ml
Buffer KAc	20 ml	20 ml
Buffer C2	50 ml	50 ml
Buffer C3	12.5 ml	12.5 ml
Wash Buffer CQW	30 ml	30 ml
Wash Buffer C5 (Concentrate)*	20 ml	20 ml
Elution Buffer CE**	15 ml	15 ml
RNase A (lyophilized)*	6 mg	6 mg
NucleoSpin® Filters L/XL (plus Collection Tubes)	20	6
NucleoSpin® Plant L/XL Columns (plus Collection Tubes)	20	6
Collection Tubes	20	6
Label for Binding Buffer C4	1	1
User Manual	1	1

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

** Composition of Elution Buffer CE: 5 mM Tris/HCl, pH 8.5

2 Product description

2.1 The basic principle

After the plant samples have been homogenized, the DNA can be extracted with lysis buffers containing chaotropic salts, denaturing agents and detergents. Lysis mixtures should be cleared by filtration using the NucleoSpin® Filters provided with the kits (alternatively by centrifugation) in order to remove polysaccharides, contaminations, and residual cellular debris. The clear flow-through is mixed with Binding Buffer C4 and ethanol to create conditions for optimal binding of DNA to the silica membrane. After loading this mixture onto the spin column, contaminants are washed away using different buffers and subsequent washing steps. The genomic DNA can finally be eluted with low salt Elution Buffer CE or water and is ready-to-use in subsequent reactions.

2.2 About this User Manual

Experienced users who are performing the isolation of genomic DNA from plant using a **NucleoSpin® Plant** isolation kit may refer to the Protocol-at-a-glance instead of this User Manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First-time users are strongly advised to read this User Manual.

2.3 Treatment of different plant samples

Plants are very heterogeneous and contain a lot of different metabolites like polyphenolics, polysaccharides or acidic components, which can lead to suboptimal extraction or subsequent processing of DNA. Therefore, we offer different lysis buffers for the optimal processing of various samples. The standard protocol ensures lysis of plant material with Buffer C1, which is based on the established CTAB procedure. Alternatively, Buffer C0 (**NucleoSpin® Plant**) and Buffer CL (**NucleoSpin® Plant L/XL**) are provided with the kit. See Table 1, which shows an overview of the plant species, which have been tested with the corresponding buffer.
Buffer CL is a SDS-based lysis system with subsequent protein precipitation by potassium acetate (KAc). This robust buffer system ensures high yields and a very good DNA quality with most common plant species.
For some species Buffer CL and Buffer C1 can be used with similar results. Choose lysis buffer according to the hints given above or in the case of not mentioned species, compare the yield with both buffers used in parallel DNA preparations to find out, if C1 or CL are better suited.
Buffer C0 is a special formulation of detergents and optimized for a wide range of species.

Table 1: Plant species which have been tested	
Buffer	**Corresponding plants**
Buffer C1	**Angiosperms (Dicotyledons):** *Arabidopsis thaliana, Brassica napus (rape), Helianthus spp. (sunflower), Hordeum vulgare (barley), Nicotiana tabacum (tobacco), Solanum tuberosum (potato), Vicia narbonensis (narbonne vetch), Pisum arvense (pea), Cerasus avium (sweet cherry)* **Angiosperms (Monocotyledons):** *Zea mays (corn), Poa spp. (grass), Allium cepa (onion), Allium sativum (garlic), Saccharum officinarum (sugar cane)*
Buffer C0	**Algae:** *Chara spec., Delesseria spec.* **Mosses:** *Funaria hygrometrica, Marchantia spec., Sphagnum spec.* **Ferns:** *Adiantum capillus-veneris, Ophioglossum spec., Salvinia auriculata* **Lycopods:** *Isoetes malinverniana, Selaginella spec.* **Gymnosperms:** *Picea abies, Pinus silvestris* **Angiosperms (Monocotyledons):** *Alisma grammineus, Echinodorus spec., Eichhornia crassipes, Miscanthus sinensis, Musa spec., Oryza sativa, Saccharum officinarum, Secale cerealis, Triticum aestivum, Tulipa spec., Zea mays*

Table 1: Plant species which have been tested	
Buffer C0	**Angiosperms** (**Dicotyledons):** *Acer pseudoplatanus, Alnus glutenosa, Arabidopsis thaliana, Arachis hypogaea, Beta vulgaris , Brassica spec., Castanea sativa, Corylus avellana, Cannabis sativa, Digitalis purpurea, Foeniculum vulgare, Geranium spec., Glycine max, Lupinus polyphyllus, Lyopersicum esculentum, Nicotiana rustica/tabacum, Phacelia tanacetifolia, Populus alba/nigra, Quercus robur, Ricinus communis, Rhododendron hort., Rhoeo discolor, Rosa spec., Sambucus nigra, Sinapis alba, Solanum tuberosum, Nymphea alba*
Buffer CL	**Angiosperms (Monocotyledons):** *Oryza sativa (rice), Zea mays (corn),* **Angiosperms** (**Dicotyledons):** *Beta vulgaris (sugar beet), Brassica napus (rape), Helianthus spp.(sunflower), Lycopersicum esculentum (tomatoe), Nicotiana tabacum (tobacco), Vicea faba (beans)*

2.4 Kit specifications

- **NucleoSpin® Plant** kits are designed for the isolation of genomic DNA from plant tissue and other biological samples like soil.
- **NucleoSpin® Plant** kits are available in 3 different sizes (see Table 2).
- **NucleoSpin® Filters L/XL** and RNase A are included in all **NucleoSpin® Plant** kits.
- Complete removal of PCR inhibitors
- The eluted DNA is ready-to-use in subsequent reactions like PCR, restriction analysis, etc.

Table 2: Kit specifications at-a-glance

Parameters	NucleoSpin® Plant (Mini)	NucleoSpin® Plant L (Midi)	NucleoSpin® Plant XL (Maxi)
Sample size	up to 100 mg	up to 250 mg	up to 10000 mg*
Typical yield	10-30 µg	20-80 µg	60-260 µg
Elution volume	100 µl	200 µl	500-2000 µl
Binding capacity	50 µg	200 µg	> 1000 µg
Time/prep	60 min	75 min	90 min
Column type	mini	midi	maxi

2.5 Storage and homogenization of samples

Plant samples can be stored frozen, in ethanol, or lyophilized. As plant tissue is very robust, the lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include grinding with a mortar and pestle in the presence of liquid nitrogen or using steel beads. We also recommend the use of any type of commercial homogenizers, e.g. bead mills. After homogenization and treatment of the sample with lysis buffer, mixtures can be cleared easily either by centrifugation or filtration with a **NucleoSpin® Filter**.

Methods to homogenize samples

- Mortar and pestle in the presence of liquid nitrogen (all sample types)

- VA steel beads (diameter: 7 mm, sample available on request): 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 fine 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.

- Commercial homogenizers

* Additional buffer volumes required, please see section 5.5, step 1.

2.6 Elution procedures

It is possible to adapt the elution method and the volume of elution buffer to the subsequent application of interest. In addition to the standard method (recovery rate about 80-90%) several modifications are possible.

- **Complete yields**: 90-100% of bound nucleic acids can be eluted by performing two elution steps with volumes as indicated in the protocols. Finally, combine eluates and determine yield.

- **High concentrated eluates**: With minimal elution volumes (at least 60% of the standard elution volume) about 70-80% of bound nucleic acids can be eluted, resulting in highly concentrated eluates.

- **Optimized elution:** Use elution buffer preheated to 70°C. After loading half of the regular elution buffer volume (e.g. 50 µl) onto the membrane, incubate the **NucleoSpin® column** for **3 min** at **70°C**. Afterwards add the remaining elution buffer (e.g. another 50 µl) and incubate further **2 min** before centrifugation resulting in optimal recovery of bound nucleic acids with standard volumes of elution buffer.

- Special recommendations for NucleoSpin® Plant XL

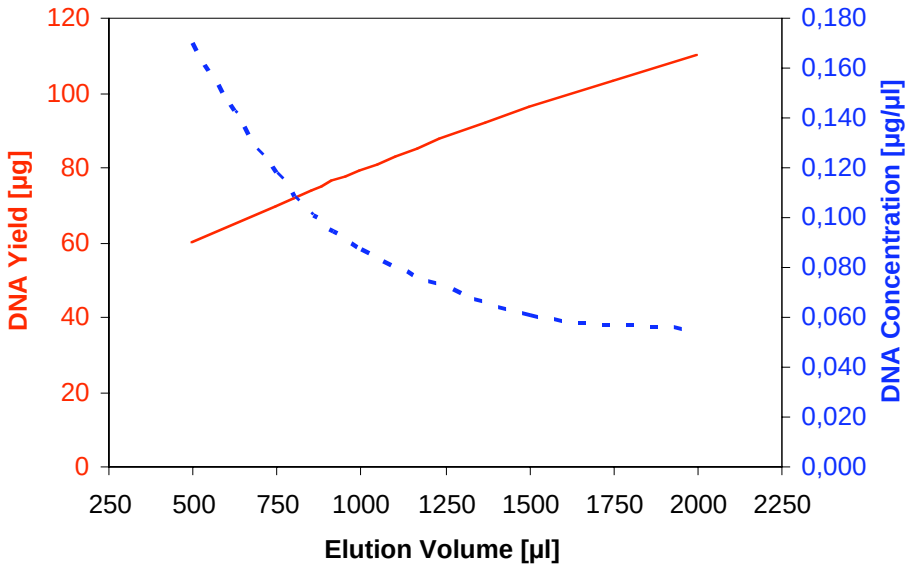


Fig. 1: Dependence of DNA yield (solid line) and concentration (dotted line) on elution volume. Genomic DNA was purified from 3 g fresh maize leaves (*Zea mays*) and eluted in final volumes of 0.5-2 ml elution buffer. If large amounts of DNA were loaded onto the NucleoSpin® Plant XL Column and lower DNA concentration is acceptable, an elution volume of 2 ml is recommended to recover DNA quantitatively. However, if highly concentrated DNA is required, smaller volumes down to 500 µl can be applied. The absolute yield will furthermore depend on the kind of plant material and sample quality (fresh, old, frozen).

3 Storage conditions and preparation of working solutions

Attention:
Buffers C0, C1-3, CW and CQW contain guanidine hydrochloride, CTAB and detergents! Wear gloves and goggles!

- All kit components can be stored at room temperature (20-25°C) and are stable for up to one year.

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

- **Wash Buffer C5:** Add the given volume of ethanol (96-100%) to **Wash Buffer C5 (Concentrate)** before use. Store Wash Buffer C5 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® Plant		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740570.10**	**740570.50**	**740570.250**
Wash Buffer C5 (Concentrate)	6 ml add 24 ml ethanol	25 ml add 100 ml ethanol	50 ml add 200 ml ethanol
RNase A	1.5 mg dissolve in 150 µl H_2O	6 mg dissolve in 600 µl H_2O	2 x 15 mg dissolve in 1500 µl H_2O each

	NucleoSpin® Plant L	**NucleoSpin® Plant XL**
	20 preps	**6 preps**
Cat. No.	**740539.20**	**740540.6**
Wash Buffer C5 (Concentrate)	20 ml add 80 ml ethanol	20 ml add 80 ml ethanol
RNase A	6 mg dissolve in 600 µl H_2O	6 mg dissolve in 600 µl H_2O

- **Lysis Buffer C0:** Add the total content of **Solution C** to **Lysis Buffer C0 Concentrate** and mix well. The resulting **Lysis Buffer C0** is stable for up to one year and should be stored at room temperature (20-25°C). Powder does not dissolve completely. Directly before use Lysis Buffer C0 should be incubated at 45°C for 10 minutes and mixed well!

- **Lysis Buffer CL**: Please note that the containing SDS may precipitate at temperatures below 20°C. In case of precipitation, incubate the bottle for several minutes at about 30-40°C and mix well until the precipitate is redissolved.

- **Binding Buffer C4**: Transfer the total contents of **Buffer C2** to **Buffer C3** and mix well. The resulting **Binding Buffer C4** is stable for up to one year at room temperature. For a better dissolving 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 small quantities of Buffer C3 and Buffer C2 in a 1:4 ratio respectively. For example, 100 µl Buffer C3 and 400 µl Buffer C2. Mix by pipetting up and down.

Reagents and equipment supplied by the user

- Ethanol (96-100%)

- Thermal heating block or water bath (NucleoSpin® Plant L/ XL)

- Centrifuge for microcentrifuge tubes (NucleoSpin® Plant) or centrifuge for 15 ml (NucleoSpin® Plant L) or 50 ml (NucleoSpin® Plant XL) centrifuge tubes, respectively

- 1.5 ml microcentrifuge tubes (NucleoSpin® Plant), 15 ml (NucleoSpin® Plant L) or 50 ml centrifuge tubes (NucleoSpin® Plant XL), respectively, for sample lysis and DNA elution

- Manual pipettors and disposable tips

- Personal protection equipment (lab coat, gloves, goggles)

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® Plant, Plant L, and Plant XL 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
C2	Guanidine hydrochloride	 Xn*	Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	
CQW	Guanidine hydrochloride + ethanol < 40%	 Xn*	Flammable. Harmful if swallowed. Irritating to eyes and skin	R 10-22-36/38	S 7-16
CW	Guanidine hydrochloride + isopropanol < 25%	 Xn*	Flammable. Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	
RNase A	RNase A, lyophilized	 Xi*	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

* 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 Protocols for DNA isolation from plant

5.1 Genomic DNA purification with NucleoSpin® Plant (Lysis Buffer C1 or C0)

1 Homogenize sample

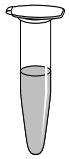
Homogenize up to 100 mg wet weight or up to 20 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.5).

homogenize samples

*Remember to preheat **Buffer C0 for 10 min at 45°C** and mix well before use.*

2 Cell lysis

Transfer the resulting powder to a new 1.5 ml microcentrifuge tube (not provided) and add **400 µl Buffer C1** or **C0**. Vortex the mixture thoroughly.



+ 400 µl C1 or C0

*If the volume of lysis buffer is not large enough, the plant powder can be resuspended in additional **Buffer C1** or **C0**. If the volume is changed, do not forget to increase the volumes of **Buffer C4** and **ethanol** proportionally (see step 4).*

*Optional: If samples contain large amounts of RNA, we recommend the addition of **10 µl RNase A** solution to the **C1/C0 lysis mixture**.*

60°C 30 min

Incubate the suspension for **30 min at 60°C**.

3 Filtration / Clarification of lysate

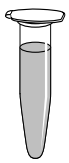
Place a **NucleoSpin® Filter** (violet ring) into a new Collection Tube (2 ml) and load the lysate onto the column. Centrifuge for **5 min at < 11,000 x *g*** and collect the clear flow-through.

5 min 11,000 x *g*

*Alternatively centrifuge the mixture for **5 min at 11,000 x g**. Transfer 300 µl of the clear lysate to a new Collection Tube.*

4 Adjust DNA binding conditions

Transfer **300 µl of the clear flow-through** into a new 1.5 ml microcentrifuge tube (not provided). Add **300 µl Buffer C4** and **200 µl ethanol**. Mix by inverting the tube 2-4 times.

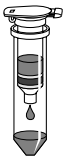


+ 300 µl C4

+ 200 µl ethanol

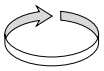
***C4** and **ethanol** may be premixed.*

5 Bind DNA



load sample

Place a NucleoSpin® Plant Column (grey ring) into a new Collection Tube (2 ml) and load sample.

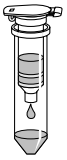


1 min
11,000 x *g*

Centrifuge for **1 min at 11,000 x *g***. Discard flow-through. Repeat this step until all of the lysate has passed through the NucleoSpin® Plant Column.

6 Wash silica membrane

1st wash

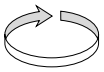


+ 400 µl CW

1 min
11,000 x *g*

Add **400 µl Buffer CW** to the NucleoSpin® Plant Column. Centrifuge for **1 min at 11,000 x *g***. Discard flow-through.

2nd wash

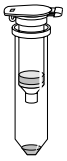


+ 700 µl C5

1 min
11,000 x *g*

Add **700 µl Buffer C5** to the NucleoSpin® Plant Column. Centrifuge for **1 min at 11,000 x *g***. Discard flow-through.

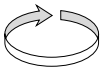
7 Wash / Dry silica membrane



+ 200 µl C5

3rd wash

Add another **200 µl Buffer C5** to the NucleoSpin® Plant Column. Centrifuge for **2 min at 11,000 x *g*** in order to remove **Buffer C5** completely and dry the silica membrane.



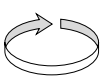
2 min
11,000 x *g*

8 Elute highly pure DNA

+ 100 µl CE (70°C)

RT
5 min

Place the NucleoSpin® Plant Column into a new 1.5 ml microcentrifuge tube (not provided). Pipette **100 µl Elution Buffer CE (preheated to 70°C)** onto the membrane. Incubate at room temperature for **5 min**. Centrifuge for **1 min at 11,000 x *g*** to elute the DNA.



1 min
11,000 x *g*

5.2 NucleoSpin® Plant Support protocol for fungi (CTAB method)

1 Homogenize sample

Wash **50-200 mg mycelium** (fresh weight) or material from a fruiting body of macro fungi in **ethanol**. Mycelium can be obtained from a liquid culture or scraped off (with or without agar) from the surface of a solid medium.

Cover sample completely with **ethanol** and mix carefully. Short washing in ethanol is sufficient in most cases, although incubation overnight sometimes increases DNA yield. (Long-term storage in ethanol is also possible).

Remove ethanol by pipetting and squeezing the mycelium.

2 Cell lysis

Place the sample in a micro-pistil. Add **150 mg siliconized glass beads** or sea sand and **200 µl Buffer C1**. Homogenize sample and vortex regularly. Add an additional **100 µl Buffer C1** and continue to homogenize the sample.

*If the volume of lysis buffer is not large enough, the powder can be resuspended in additional **300 µl Buffer C1**. If the volume is changed, do not forget to increase the volumes of **Buffer C4** and **ethanol** proportionally (step 4).*

*Optional: If a high RNA or protein content is present, we recommend to add **10 µl RNase A and/or Proteinase K** solution (5-10 mg/ml stock solutions) to the **C1 lysis solution** in order to minimize any contaminants (Proteinase K; see ordering information).*

Incubate for **30-60 min at 65°C.**

Add **1 volume chloroform:isoamylalcohol (24:1)**. Vortex for **10 sec** and separate phases by centrifugation for **10 min at 20,000 x *g***. Pipette the top aqueous layer into a new 1.5 ml microcentrifuge tube (not provided).

The chloroform extraction step is optional but highly recommended.

3 Filtration / Clarification of lysate

Place a **NucleoSpin® Filter** (violet ring) into a new Collection Tube (2 ml) and load the lysate onto the column. Centrifuge for **5 min at < 11,000 x *g*** and collect the clear flow-through.

*Alternatively centrifuge the mixture for **5 min at 11,000 x g**. Transfer 300 µl of the clear lysate to a new tube (not provided).*

4 Adjust DNA binding conditions

Transfer **300 µl of the clear flow-through** into a new 1.5 ml microcentrifuge tube (not provided). Add **300 µl Buffer C4** and **200 µl ethanol**. Vortex the mixture for 30 sec.

C4 *and* ***ethanol*** *may be premixed.*

5 Bind DNA

Place a NucleoSpin® Plant Column (grey ring) in a new Collection Tube (2 ml). Pipette the mixture onto the NucleoSpin® Plant Column. Centrifuge for **1 min at 11,000 x *g***. Discard flow-through.

Proceed with step 6 of the standard protocol (see section 5.1).

5.3 NucleoSpin® Plant Support protocol for soil, compost, dung, and animal excrements (CTAB method)

Attention: Additional equipment necessary

- Bead mill (e.g. Pulverisette 0, Fritsch - Idar-Oberstein), or mortar and pestle
- Sea sand (siliconized)
- Extraction buffer (not included): 2 M NaCl, 20 mM EDTA, 100 mM Tris-Cl, 2% (w/v) CTAB, 2% (w/v) Polyvinylpyrrolidon (MW 40,000), pH 8.0

1 Homogenize sample

Weigh **5 g soil or 2 g dung** into a petri dish. Add extraction buffer until the sample is completely soaked. Heat the sample in a **microwave oven** (400 W) for a few seconds until the extraction buffer is foaming.

Extraction buffer may be added to keep the sample in a slushy state.

2 Cell lysis

Transfer sample into a bead mill or mortar. Add **0.5 ml sea sand** and disrupt the sample.

3 Filtration/Clarification of lysate

Transfer the homogenized sample into a centrifuge tube (e.g. Sorvall SS34) and centrifuge for **10 min at 5,000 x *g***. Pipette **300 µl** of the clear supernatant into a new 1.5 ml centrifuge tube (not provided).

4 Adjust DNA binding conditions

Add **300 µl Buffer C4** and **200 µl ethanol**. Vortex the mixture for 30 sec.

5 Bind DNA

Place a NucleoSpin® Plant Column into a new Collection Tube (2 ml) and pipette the mixture onto the NucleoSpin® Plant Column. Centrifuge for **1 min at 11,000 x *g***. Discard flow-through.

Proceed with step 6 of the standard protocol (see section 5.1)

5.4 Genomic DNA purification with NucleoSpin® Plant L (Lysis Buffer C1 or CL)

For centrifugation, a centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,000 - 4,500 x *g* is required.

1 Homogenize sample

Homogenize plant sample (for homogenization methods see section 2.5)

homogenize samples

Use 250 mg wet weight or up to 50 mg dry weight (lyophilized samples).

2a) Cell lysis using Buffer C1

Transfer the resulting powder to a new tube (not provided) and add **1.25 ml Buffer C1 per 250 mg plant sample**. For dry samples larger volumes of Buffer C1 may be necessary if lysis mixture is too viscous.

+ 1.25 ml C1

+ 20 µl RNase A

Add **20 µl RNase A solution**/250 mg sample. Incubate the suspension for **30 min at 60°C.**

60°C 30 min

Proceed with step 3.

2b) Cell lysis using Buffer CL

+ 1.6 ml CL

Transfer the resulting powder to a new tube (not provided) and add **1.6 ml Buffer CL per 250 mg plant sample**. For dry samples larger volumes of Buffer CL may be necessary if lysis mixture is too viscous.

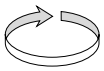
+ 20 µl RNase A

+ 300 µl β-ME

Add **20 µl RNase A solution**/250 mg sample and 300 µl β-mercaptoethanol.

60°C 30 min

Incubate the suspension for **30 min at 60°C.**



+ 0.5 ml KAc

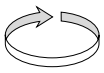
Afterwards add **0.5 ml Buffer KAc** and incubate for **5 min on ice**. Centrifuge the mixture for **5 min at 4,500 x *g*** and load the supernatant onto a NucleoSpin® Filter L as described in step 3.

0°C 5 min

5 min 4,500 x *g*

3 Filtration/Clarification of lysate

Load the suspension or the pre-cleared supernatant onto a NucleoSpin® Filter L and centrifuge the mixture for **10 min at 4,500 x *g***. Transfer clear flow-through (do not disturb any particle-pellet) in a 15 ml centrifuge tube (not provided) and determine the volume.



10 min
4,500 x *g*

4 Adjust DNA binding conditions

Add **1 vol Buffer C4 and 1 vol ethanol** to the cleared lysate. Vortex the mixture immediately for **30 sec**.

+ 1 vol C4

+ 1 vol ethanol

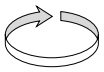
vortex 30 sec

5 Bind DNA

Take a NucleoSpin® Plant L Column placed in a Collection Tube and load sample onto the NucleoSpin® Plant L Column.

load sample

Centrifuge for **2 min at 4,500 x *g***. Discard flow-through.



2 min
4,500 x *g*

6 Wash silica membrane

1st wash

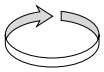
Pipette **1 ml Buffer CQW** onto the NucleoSpin® Plant L Column. Centrifuge for **2 min at 4,500 x *g***.

+ 1 ml CQW

+ 2 ml C5

2nd wash

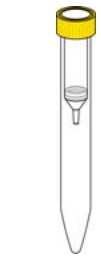
Pipette **2 ml Buffer C5** onto the NucleoSpin® Plant L Column. Centrifuge for **2 min at 4,500 x *g***. Discard flow-through.

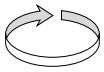


2 min
4,500 x *g*

7 Dry silica membrane

Place the NucleoSpin® Plant L Column in the emptied Collection Tube and centrifuge for **10 min at 4,500 x *g*** in order to remove Buffer C5 completely.





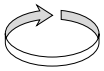
10 min
4,500 x *g*

8 Elute highly pure DNA

Place the NucleoSpin® Plant L Column in a new Collection Tube (15 ml). Pipette **200 µl Elution Buffer CE** (preheated to 70°C) onto the membrane. Incubate for **5 min at room temperature**. Centrifuge for **3 min at 4,500 x *g*** to collect the DNA and **repeat elution with 100-200 µl**. Combine eluates for downstream processes.

+ 200 µl CE
(70°C)

RT
5 min



3 min
4,500 x *g*

5.5 Genomic DNA purification with NucleoSpin® Plant XL (Lysis Buffer C1 or CL)

For centrifugation, a centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,000 - 4,500 x *g* is required.

1 Homogenize sample

Homogenize plant sample (for homogenization methods see section 2.5)

Use up to 10000 mg wet weight or up to 2000 mg dry weight (lyophilized samples).

homogenize samples

Note: The volumes of buffers included in the kit are adapted to an average application of up to 1000 mg wet weight. For plant samples > 1000 mg, additional RNase A, Buffer C1 or Buffer CL (and Buffer KAc), as well as Buffer C4 are required. Please see ordering information.

2a) Cell lysis using Buffer C1

Transfer the resulting powder to a new tube (not provided) and add **5 ml Buffer C1 per 1000 mg plant sample**. For dry samples larger volumes of Buffer C1 may be necessary if lysis mixture is too viscous.

Add 80 µl RNase A solution/1000 mg sample.

Incubate the suspension for **30 min at 60°C.**

Proceed with step 3.

+ 5 ml C1

+ 80 µl RNase A

60°C, 30 min

2b) Cell lysis using Buffer CL

Transfer the resulting powder to a new tube (not provided) and add **8 ml Buffer CL per 1000 mg plant sample**. For dry samples larger volumes of Buffer CL may be necessary if lysis mixture is too viscous.

Add 80 µl RNase A solution/1000 mg sample and 1.2 ml β-mercaptoethanol.

Incubate the suspension for **30 min at 60°C.**

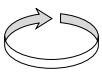
Afterwards add **2.5 ml Buffer KAc** and incubate for **5 min on ice**. Centrifuge the mixture for **5 min at 4,000 x *g*** and load the supernatant onto a NucleoSpin® Filter XL as described in step 3.

+ 8 ml CL

+ 80 µl RNase A

+ 1.2 ml β-ME

60°C, 30 min



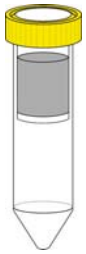
+ 2.5 ml KAc

0°C, 5 min

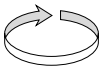
5 min, 4,000 x *g*

3 Filtration/Clarification of lysate

Note: If a large volume of lysate has to be cleared, a filtration step using e.g. NucleoBond® Folded Filters (not provided, see ordering information) can be performed before loading the lysate to the NucleoSpin® Filter XL. The latter step can even be omitted if the resulting filtrate is absolutely clear.

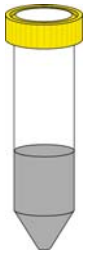


10 min
4,000 x *g*



Load suspension or pre-cleared supernatant onto a **NucleoSpin® Filter XL** and centrifuge the mixture for **10 min at 4,000 x *g***. Transfer clear flow-through (do not disturb any particle-pellet) to a 50 ml centrifuge tube (not provided) and determine the volume.

4 Adjust DNA binding conditions



+ 1 vol C4

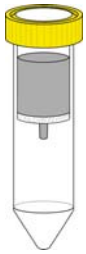
+ 1 vol ethanol

vortex 30 sec

Add **1 vol Buffer C4 and 1 vol ethanol** to the cleared lysate. Vortex the mixture immediately for **30 sec**.

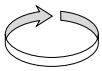
5 Bind DNA

Take a NucleoSpin® Plant XL Column placed in a Collection Tube and load up to 15 ml of the mixture onto the NucleoSpin® Plant XL Column.



load sample

2 min
4,000 x *g*

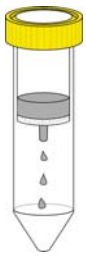


Centrifuge for **2 min at 4,000 x *g***. Discard flow-through.

Discarding the first flow-through may be omitted. Be careful after the second loading step during removal of the tube from the centrifuge and the removal of the column from the tube: keep tube with column upright to minimize contact of flow-through with the column outlet.

If more than 30 ml need to be loaded onto the column, discard flow-through and repeat the loading procedure as often as necessary.

6 Wash silica membrane



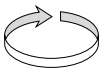
+ 4 ml CQW

2 min
4,000 x *g*

1st wash

Pipette **4 ml Buffer CQW** onto the NucleoSpin® Plant XL Column. Centrifuge for **2 min at 4,000 x *g***.

+ 10 ml C5



15 min
4,000 x *g*

2nd wash

Pipette **10 ml Buffer C5** onto the NucleoSpin® Plant XL Column. Centrifuge for **15 min at 4,000 x *g***. Discard flow-through.

7 Dry silica membrane

The **drying** of the NucleoSpin® Plant XL Column is performed by prolonged centrifugation time (15 min) in the 2nd wash step.

8 Elute highly pure DNA

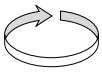
Note: Refer to section 2.6, Fig. 1 to select most appropriate volume of Elution Buffer CE (e.g. 500-2000 µl).

+ 1000 µl CE (70°C)

RT, 5 min

3 min
4,000 x *g*

Place the NucleoSpin® Plant XL Column into a new Collection Tube (50 ml). Pipette half of the final volume of Elution Buffer CE (e.g. 1000 µl, preheated to 70°C) onto the membrane. Incubate for **5 min at room temper-ature**. Centrifuge for **3 min at 4,000 x *g*** to collect the DNA.



repeat elution with fresh buffer

Repeat elution step a second time with the same volume fresh Buffer CE (e.g. 1000 µl, preheated to 70°C) to elute DNA completely.

For alternative elution procedures see section 2.6.

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 2.5). 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. • Lyophilizing the material can also circumvent this problem. In this case grinding of the material becomes easier. *Suboptimal lysis buffer used* • From our tests on numerous plant species, Lysis Buffer C0 and C1 generate different yields. *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). *Sample contains too much RNA* • Add RNase A solution as indicated in the protocol to the lysis buffer before heat incubation. If this is not successful, add the enzyme to the cleared lysate and incubate for 30 min at 37°C. *Suboptimal Elution* • The DNA can either be eluted in higher volumes or by repeating the elution step up to three times. Remember to preheat elution buffer to 70°C prior to elution. • Also check the pH of the elution buffer used, which should be in the range of pH 8.0 - 8.5. To ensure correct pH, use supplied Elution Buffer CE.

Problem	Possible cause and suggestions
DNA is degraded	*Sample was contaminated with DNase* • Preheat elution buffer to 70°C for 5 min to eliminate any possible DNase contamination. This precaution is not necessary for buffers supplied by MACHEREY-NAGEL, which are delivered free of RNase and DNase. *Centrifugation speed was too high* • Centrifuge at a maximum speed of 11,000 x *g*. Higher velocities may lead to shearing of the DNA.
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 may disturb subsequent reactions. Use of water or supplied Elution Buffer CE (free of EDTA) is highly recommended.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® Plant	740570.10/ .50/ .250	10/ 50/ 250 preps
NucleoSpin® Plant L	740539.20	20 preps
NucleoSpin® Plant XL	740540.6	6 preps
Buffer C1	740930	100 ml
Buffer CL, Buffer KAc	on request	
Buffer C4 (100 ml C2 + 25 ml C3)	740935	125 ml
Buffer C5 Concentrate (for 100 ml Buffer C5)	740931	20 ml
Buffer CW	740932	100 ml
Collection Tubes (2 ml)	740600	1000
NucleoSpin® Filters	740606	50
NucleoBond® Folded Filters	740561	50
RNase A	740505	100 mg
RNase A	740505.50	50 mg

6.3 Product use restriction / warranty

NucleoSpin® Plant kits components were developed, designed, distributed and sold **for RESEARCH PURPOSES ONLY**. They are suitable ***for IN VITRO USES only***. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).
It is rather the responsibility of the user to verify the use of the **NucleoSpin® Plant** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.
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