



Genomic DNA from Plant

User Manual

NucleoSpin® Plant II

NucleoSpin® Plant II Midi

NucleoSpin® Plant II Maxi

March 2010/Rev.04

Genomic DNA Purification from Plant

Protocol-at-a-glance (Rev.04)
















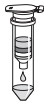












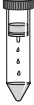








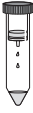

| NucleoSpin® Plant II | | Mini | Midi | Maxi |
|---|---|---|--|------|
| 1 Homogenize samples |  100 mg |  400 mg |  1500 mg | |
| 2 Cell lysis |  400 µl PL1 10 µl RNase A 65°C, 10 min |  1.7 ml PL1 25 µl RNase A 65°C, 10 min |  6 ml PL1 100 µl RNase A 65°C, 10 min | |
| | ALTERNATIVELY  300 µl PL2 10 µl RNase A 65°C, 10 min 75 µl PL3 0°C, 5 min | ALTERNATIVELY  1.5 ml PL2 25 µl RNase A 65°C, 10 min 200 µl PL3 0°C, 5 min | ALTERNATIVELY  5.3 ml PL2 100 µl RNase A 65°C, 10 min 700 µl PL3 0°C, 5 min | |
| 3 Filtration / Clarification of lysate |   ≥ 11,000 x g 2 min |   4,500 x g 10 min |   4,500 x g 10 min | |
| 4 Adjust DNA binding conditions | 450 µl PC | 2.3 ml PC | 8 ml PC | |
| 5 Bind DNA |   ≥ 11,000 x g 1 min |   4,500 x g 2 min |   4,500 x g 2 min | |
| 6 Wash and dry silica membrane | 1st 400 µl PW1  ≥ 11,000 x g 1 min | 1st 1 ml PW1  4,500 x g 2 min | 1st 4 ml PW1  4,500 x g | |
| | 2nd 700 µl PW2   ≥ 11,000 x g 1 min | 2nd 3 ml PW2   4,500 x g 2 min | 2nd 10 ml PW2   4,500 x g 2 min | |
| | 3rd 200 µl PW2  ≥ 11,000 x g 2 min | 3rd 1 ml PW2  4,500 x g 10 min | 3rd 2 ml PW2  4,500 x g 10 min | |
| 7 Elute DNA |  50 µl PE 70°C, 5 min  ≥ 11,000 x g 1 min Repeat elution step |  200 µl PE 70°C, 5 min  4,500 x g 2 min Repeat elution step |  1000 µl PE 70°C, 5 min  4,500 x g 2 min Repeat elution step | |

Table of contents

| | | |
|-----|---|----|
| 1 | Components | 4 |
| 1.1 | Kit contents | 4 |
| 1.2 | Reagents, consumables, and equipment to be supplied by user | 6 |
| 1.3 | About this User Manual | 6 |
| 2 | Product description | 7 |
| 2.1 | The basic principle | 7 |
| 2.2 | Kit specifications | 7 |
| 2.3 | Storage of plant samples | 8 |
| 2.4 | Homogenization of plant samples | 8 |
| 2.5 | Lysis of plant samples | 9 |
| 2.6 | Elution procedures | 11 |
| 3 | Storage conditions and preparation of working solutions | 13 |
| 4 | Safety instructions – risk and safety phrases | 14 |
| 5 | NucleoSpin® Plant II protocols | 15 |
| 5.1 | Standard protocol for genomic DNA from plant | 15 |
| 5.2 | Support protocol for genomic DNA from fungi | 18 |
| 5.3 | Support protocol for soil, compost, dung, and animal excrements | 19 |
| 6 | NucleoSpin® Plant II Midi protocol | 20 |
| 7 | NucleoSpin® Plant II Maxi protocol | 23 |
| 8 | Appendix | 26 |
| 8.1 | Troubleshooting | 26 |
| 8.2 | Ordering information | 28 |
| 8.3 | Product use restriction/warranty | 29 |

1 Components

1.1 Kit contents

| NucleoSpin® Plant II | | | |
|---|-----------------------|-----------------------|-------------------------|
| Cat. No. | 10 preps 740770.10 | 50 preps 740770.50 | 250 preps 740770.250 |
| Lysis Buffer PL1 | 5 ml | 25 ml | 125 ml |
| Lysis Buffer PL2 | 4 ml | 20 ml | 100 ml |
| Precipitation Buffer PL3 | 1 ml | 5 ml | 25 ml |
| Binding Buffer PC | 6 ml | 30 ml | 125 ml |
| Wash Buffer PW1 | 6 ml | 30 ml | 125 ml |
| Wash Buffer PW2 (Concentrate)* | 6 ml | 25 ml | 50 ml |
| Elution Buffer PE** | 5 ml | 15 ml | 30 ml |
| RNase A (lyophilized)* | 1.5 mg | 6 mg | 2 x 15 mg |
| NucleoSpin® Filters (violet rings) | 10 | 50 | 250 |
| NucleoSpin® Plant II Columns (green rings) | 10 | 50 | 250 |
| Collection Tubes (2 ml) | 20 | 100 | 500 |
| User Manual | 1 | 1 | 1 |

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

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

1.1 Kit contents *continued*

| Cat. No. | NucleoSpin® Plant II Midi | NucleoSpin® Plant II Maxi |
|--|---------------------------|---------------------------|
| | 20 preps 740771.20 | 10 preps 740772.10 |
| Lysis Buffer PL1 | 2 x 25 ml | 75 ml |
| Lysis Buffer PL2 | 2 x 20 ml | 60 ml |
| Precipitation Buffer PL3 | 5 ml | 15 ml |
| Binding Buffer PC | 2 x 30 ml | 125 ml |
| Wash Buffer PW1 | 30 ml | 50 ml |
| Wash Buffer PW2 (Concentrate)* | 25 ml | 50 ml |
| Elution Buffer PE** | 15 ml | 30 ml |
| RNase A (lyophilized)* | 6 mg | 10 mg |
| NucleoSpin® Filters L/XL (plus Collection Tubes) | 20 | 10 |
| NucleoSpin® Plant II Midi/Maxi Columns (plus Collection Tubes) | 20 | 10 |
| Collection Tubes (15 ml/50 ml) | 20 | 10 |
| User Manual | 1 | 1 |

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

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

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100% ethanol

Consumables

- 1.5 ml microcentrifuge tubes (**NucleoSpin® Plant II**) or 15/50 ml tubes (**NucleoSpin® Plant II Midi/Maxi**)
- Disposable tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes (**NucleoSpin® Plant II**) or an appropriate centrifuge with swing-out rotors capable of reaching 4,500 x *g* for 15 ml/50 ml tubes (**NucleoSpin® Plant II Midi/Maxi**)
- Thermal heating-block or water bath for incubation and preheating of Elution Buffer PE (to 70°C)
- Equipment for sample disruption and homogenization (see section 3.3)
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this User Manual

It is strongly recommended reading the detailed protocol sections of this User Manual if the **NucleoSpin® Plant II/Midi/Maxi** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at www.mn-net.com.

2 Product description

2.1 The basic principle

The plant samples are homogenized by mechanical treatment. Then the DNA can be extracted with Lysis Buffers PL1 or PL2 containing chaotropic salts, denaturing agents, and detergents. Crude lysates should be cleared by centrifugation and/or filtration using the **NucleoSpin® Filters** provided with the kits in order to remove polysaccharides, contaminations, and residual cellular debris. The clear flow-through is mixed with Binding Buffer PC 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 Wash Buffers PW1 and PW2. The genomic DNA can finally be eluted with low salt Elution Buffer PE (5 mM Tris/HCl, pH 8.5) or nuclease-free water and is ready-to-use for subsequent reactions.

2.2 Kit specifications

- **NucleoSpin® Plant II** kits are designed for the isolation of genomic DNA from plant tissue and other biological samples like soil using two optimized lysis buffer systems based on the established CTAB and SDS methods.
- **NucleoSpin® Filters** are included for conveniently clearing the crude lysates.
- RNase A is included to remove RNA and to allow photometric quantification of pure genomic DNA.
- The optimized Binding Buffer PC and the optional chaotropic Wash Buffer PW1 completely remove proteins, RNA, metabolites, and other PCR inhibitors.
- The eluted DNA is ready-to-use for subsequent reactions like PCR, restriction analysis, Southern Blotting etc.

Table 1: Kit specifications at a glance

| Parameter | NucleoSpin® Plant II | NucleoSpin® Plant II Midi | NucleoSpin® Plant II Maxi |
|------------------|---|---|---|
| Sample size | Up to 100 mg wet weight Up to 20 mg dry weight | Up to 400 mg wet weight Up to 80 mg dry weight | Up to 1500 mg wet weight Up to 300 mg dry weight |
| Typical yield | 1 – 30 µg | 10 – 100 µg | 50 – 300 µg |
| Elution volume | 100 µl | 400 µl | 2000 µl |
| Binding capacity | 50 µg | 200 µg | > 500 µg |
| Time/prep | 30 min | 90 min | 90 min |

2.3 Storage of plant samples

Plant samples can be stored in ethanol, lyophilized, or frozen. Fresh material can be kept at 4°C for one day but should be frozen at -20°C for longer storage.

2.4 Homogenization of plant samples

As plant tissue is very robust, the lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills using steel or glass beads. However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields.

After homogenization and treatment of the sample with lysis buffer, the crude lysate can be cleared easily either with **NucleoSpin® Filters** or by centrifugation.

Methods to homogenize samples

- Grinding with mortar and pestle in the presence of liquid nitrogen: Freeze plant material in liquid nitrogen and do not let the sample thaw at any time during homogenization. Precool mortar and pestle using liquid nitrogen. Grind frozen sample thoroughly to a fine powder and refill mortar occasionally with liquid nitrogen to keep the sample frozen. Use a precooled spatula to transfer the sample in precooled tubes. Make sure no liquid nitrogen is transferred or all nitrogen has evaporated before closing the tube.
- VA steel beads (diameter: 7 mm, sample available on request): Put 4 – 5 beads and plant material into a 15 ml plastic tube (Falcon), chill the tube in liquid nitrogen and vortex for about 30 seconds (e.g., with a Multi Pulse Vortexer, Schütt Labortechnik GmbH, www.schuett-labortechnik.de). Repeat the 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 using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube since this leads to sticking and loss of plant material attached to the beads.
- Rotor-stator homogenizers are only useful to disrupt soft plants in the presence of lysis buffer. Keep homogenizer submerged at all times to reduce foaming.

2.5 Lysis of plant samples

Increasing the amount of starting material

The standard protocols of **NucleoSpin® Plant II/Midi/Maxi** kits allow processing of 10 – 1500 mg of plant material. This usually yields 1 – 300 µg of high quality DNA. However, the amount of DNA that can be expected per mg of sample depends on the size and ploidy of the genome. For example 100 mg fresh wheat with a hexaploid genome (1.7×10^{10} bp) contains 30 µg DNA, whereas the same amount of Arabidopsis with a smaller diploid genome (1.9×10^8 bp) only yields 3 µg DNA.

Thus, it might be advantageous to process even more than the recommended sample mass (up to 5-fold) to obtain a reasonable DNA yield. However, to ensure a complete lysis, all lysis buffer volumes of protocol step 2 have to be increased proportionally and multiple loading steps are necessary. Additional lysis buffers (PL1, PL2, PL3, RNase) have to be purchased separately (see ordering information).

Choosing the optimal lysis buffer system

Plants are very heterogeneous and contain varying amounts of polyphenols, acidic components, or polysaccharides which can lead to suboptimal DNA extraction or performance in downstream applications. Therefore, we offer two different lysis buffers for the optimal processing, high yields, and an excellent DNA quality with most common plant species.

The standard protocol uses Lysis Buffer PL1, which is based on the established CTAB procedure. Additionally, the SDS based Lysis Buffer PL2 is provided which requires subsequent protein precipitation by potassium acetate (Precipitation Buffer PL3). For some plant species Lysis Buffers PL1 and PL2 can be used with similar results. However, for most plant material the lysis efficiency is different due to the negative charge of SDS and the positive charge of CTAB.

Table 2 gives an overview about customer data on different plant species and the corresponding buffer system that has been tested successfully using **NucleoSpin® Plant II**. **Important! For a large variety of plant species both lysis buffers allow good results.**

Use the table only for a rough orientation and guideline which buffer system has already been tested. In order to find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with both lysis buffers.

Table 2: Plant species tested with NucleoSpin® Plant II

| Plant species | Plant tissue/organ | Lysis buffer successfully tested | |
|---|--|----------------------------------|------------|
| | | PL1 | PL2 |
| <i>Abies alba</i> (fir) | Needle | ✓ | ✓ |
| <i>Amorphophallus titanum</i> | Leaf | ✓ | Not tested |
| <i>Apium graveolens</i> (celery) | Corm | ✓ | ✓ |
| <i>Arabidopsis thaliana</i> | Leaf | ✓ | Not tested |
| <i>Boreava orientalis</i> | Leaf, herbarium sample | ✓ | ✓ |
| <i>Cleisostoma racemiferum</i> | Inflorescence rachis, silica-gel dried | ✓ | Not tested |
| <i>Doritis pulcherrima</i> | Leaf, silica-gel dried | ✓ | Not tested |
| <i>Eichornia azurea</i> | Leaf | ✓ | Not tested |
| <i>Encephalartos natalensis</i> | Leaf | ✓ | Not tested |
| <i>Galium aparine</i> | Leaf | ✓ | ✓ |
| <i>Hordeum</i> sp. (barley) | Leaf | ✓ | ✓ |
| <i>Isatis kotschyana</i> | Leaf, herbarium sample | ✓ | ✓ |
| <i>Laurus azorica</i> (laurel) | Leaf | ✓ | Not tested |
| <i>Lupinus</i> sp. (lupin) | Leaf | ✓ | ✓ |
| <i>Lycopersicon esculentum</i> (tomato) | Stem | ✓ | ✓ |
| <i>Myagrum perfoliatum</i> | Leaf, herbarium sample | ✓ | ✓ |
| <i>Oryza sativa</i> (rice) | Leaf | ✓ | ✓ |
| <i>Persea feru./caerulea</i> | Leaf | ✓ | Not tested |
| <i>Pteridium</i> sp. | Leaf | ✓ | Not tested |
| <i>Pterocarya fraxinifolia</i> | Leaf | ✓ | Not tested |
| <i>Rosa</i> sp. (rose) | Leaf | ✓ | ✓ |
| <i>Rubus fruticosus</i> (blackberry) | Leaf | ✓ | ✓ |
| <i>Sameraria nummularia</i> | Leaf, herbarium sample | ✓ | ✓ |
| <i>Secale</i> sp. (rye) | Leaf | ✓ | ✓ |
| <i>Stereochilus</i> sp. | Leaf, silica-gel dried | ✓ | Not tested |
| <i>Tauscheria lasiocarpum</i> | Leaf, herbarium sample | ✓ | ✓ |
| <i>Trachycarpus takil</i> | Leaf | ✓ | Not tested |
| <i>Trichoglottis</i> sp. | Leaf, silica-gel dried | ✓ | Not tested |
| <i>Triticum aestivum</i> (wheat) | Leaf | ✓ | ✓ |
| <i>Vigna radiata</i> (mung bean) | Root | ✓ | ✓ |
| <i>Zea mays</i> (maize) | Leaf | ✓ | ✓ |
| <i>Zea mays</i> (maize) | Grain, dried, ground coarsely | ✓ | ✓ |
| Fungal mycel (not specified) | | ✓ | Not tested |
| Green algae (not specified) | | ✓ | Not tested |

2.6 Elution procedures

The following graphs show DNA yields (solid line) and the resulting DNA concentrations (dotted line) in dependence on elution buffer volume. Elution is done with either one elution step (A) or two subsequent elution steps with the indicated elution buffer volume each (B).

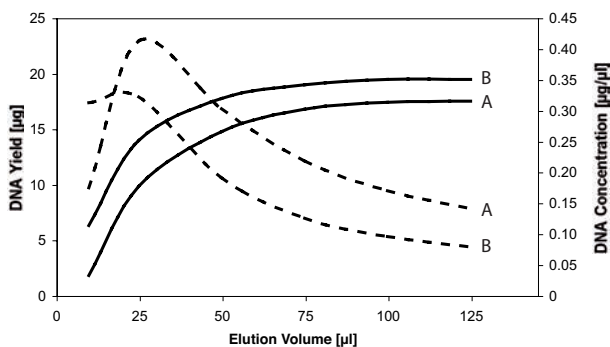


Figure 1: NucleoSpin® Plant II elution profile

Genomic DNA from 100 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 10 – 125 µl Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

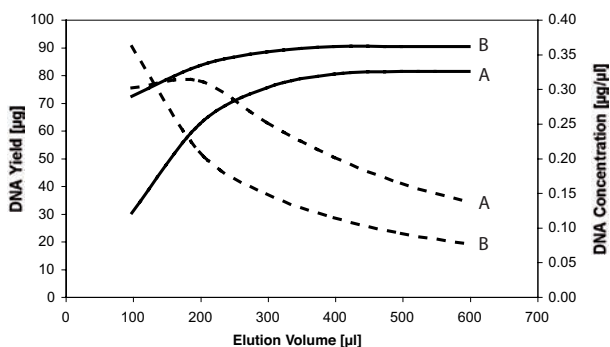


Figure 2: NucleoSpin® Plant II Midi elution profile

Genomic DNA from 400 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 100 – 600 µl Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

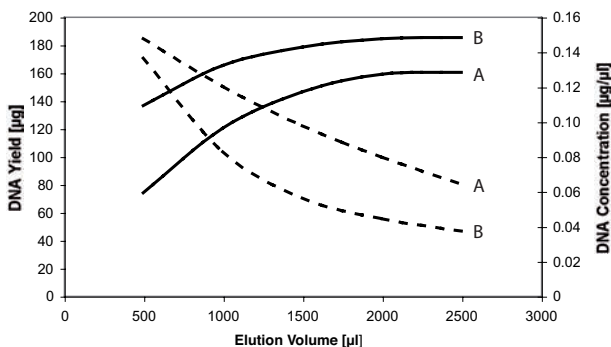


Figure 3: NucleoSpin® Plant II Maxi elution profile

Genomic DNA from 1000 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 500 – 2500 µl Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

A two-fold elution generally yields more DNA than just one elution with the same total buffer volume. This is most important for small buffer volumes. However, large volumes or eluting two times results in a lower DNA concentration.

The standard elution procedure is already optimized to yield 80 – 90% by eluting two-fold at elevated temperatures. However, if even higher yields, a higher concentration, or maximum speed is required, the elution procedure can be adapted:

Table 3: Elution parameters

| Procedure (% of exp. yield) | NucleoSpin® Plant II | NucleoSpin® Plant II Midi | NucleoSpin® Plant II Maxi |
|--------------------------------|--------------------------------|--------------------------------|----------------------------------|
| Standard elution (85 – 90%) | 50 µl + 50 µl 70°C, 5 min | 200 µl + 200 µl 70°C, 5 min | 1000 µl + 1000 µl 70°C, 5 min |
| Maximum yield (95 – 100%) | 100 µl + 100 µl 70°C, 5 min | 400 µl + 400 µl 70°C, 5 min | 2000 µl + 2000 µl 70°C, 5 min |
| High concentration (75%) | 25 µl + 25 µl 70°C, 5 min | 100 µl + 100 µl 70°C, 5 min | 500 µl + 500 µl 70°C, 5 min |
| Fast elution (60 – 70%) | 100 µl RT/70°C, 1 – 5 min | 400 µl RT/70°C, 1 – 5 min | 2000 µl RT/70°C, 1 – 5 min |

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!

All kit components can be stored at room temperature (18 – 25°C) and are stable up to one year.

Before starting any **NucleoSpin® 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 re-dissolved completely.
- **Wash Buffer PW2:** Add the given volume of ethanol (96 – 100%) as indicated on the bottle or in the table below to **Buffer PW2 Concentrate** before first use. Mark the label of the bottle to indicate that the ethanol is added. Buffer PW2 at is stable at room temperature (18 – 25°C) for at least one year.
- **RNase A:** Add the given volume of water as indicated on the vial and in the table 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 II | | | |
|-------------------------------|--|--|---|
| Cat. No. | 10 preps 740770.10 | 50 preps 740770.50 | 250 preps 740770.250 |
| Wash Buffer PW2 (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 ₂ O | 6 mg dissolve in 600 µl H ₂ O | 2 x 15 mg dissolve in 1500 µl H ₂ O each |

| NucleoSpin® Plant II Midi | | NucleoSpin® Plant II Maxi | |
|-------------------------------|---|---|--|
| Cat. No. | 20 preps 740771.20 | 10 preps 740772.10 | |
| Wash Buffer PW2 (Concentrate) | 25 ml add 100 ml ethanol | 50 ml add 200 ml ethanol | |
| RNase A | 6 mg dissolve in 600 µl H ₂ O | 10 mg dissolve in 1100 µl H ₂ O | |

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® 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 source of ignition - No Smoking! |
| S 22 | Do not breathe dust |
| S 24 | Avoid contact with the skin |
| S 25 | Avoid contact with the eyes |

* Hazard labeling not necessary if quantity per bottle below 25 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

**Hazard labeling not necessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 NucleoSpin® Plant II protocols

5.1 Standard protocol for genomic DNA from plant

Before starting the preparation:

- Check if Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 70°C.

Note: The NucleoSpin® Plant II kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

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 2.4).



Homogenize samples

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2a Cell lysis using Buffer PL1

Transfer the resulting powder to a new tube and add **400 µl Buffer PL1**. Vortex the mixture thoroughly.

+ 400 µl PL1

Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of **RNase A** (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.



Add **10 µl RNase A** solution and mix sample thoroughly.

**+ 10 µl
RNase A**

Incubate the suspension for **10 min** at **65°C**.

**65°C
10 min**

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2b Cell lysis using Buffer PL2

Transfer the resulting powder to a new tube and add **300 µl Buffer PL2**. Vortex the mixture thoroughly.

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of **RNase A**, **Buffer PL3** (step 2b), and **Buffer PC** (step 4) have to be increased proportionally.*

Add **10 µl RNase A** solution and mix sample thoroughly.

Incubate the suspension for **10 min at 65°C**.

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Add **75 µl Buffer PL3**, mix thoroughly and incubate for **5 minutes on ice** to precipitate SDS completely.

Proceed with step 3.

+ 300 µl PL2

+ 10 µl
RNase A

65°C
10 min

+ 75 µl PL3
0°C
5 min

**3 Filtration / Clarification of crude lysate**

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

If not all liquid has passed the filter, repeat the centrifugation step.

If a pellet is visible in the flow-through, transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (not provided).

Alternatively, centrifuge the crude lysate for 5 min at 11,000 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter to remove solid particles completely.



11,000 x g
2 min

4 Adjust DNA binding conditions

Add **450 µl Buffer PC** and mix thoroughly by pipetting up and down (5 times) or by vortexing.



+ 450 µl PC

5 Bind DNA

Place a **NucleoSpin® Plant II Column** (green ring) into a new Collection Tube (2 ml) and load a maximum of 700 µl of the sample.

Centrifuge for **1 min** at **11,000 x g** and discard the flow-through.

The maximum loading capacity of the NucleoSpin® Plant II Column is 700 µl. For higher sample volumes repeat the loading step.



Load lysate



11,000 x g
1 min

6 Wash and dry silica membrane

1st wash

Add **400 µl Buffer PW1** to the NucleoSpin® Plant II Column. Centrifuge for **1 min** at **11,000 x g** and discard flow-through.

2nd wash

Add **700 µl Buffer PW2** to the NucleoSpin® Plant II Column. Centrifuge for **1 min** at **11,000 x g** and discard flow-through.

3rd wash

Add another **200 µl Buffer PW2** to the NucleoSpin® Plant II Column. Centrifuge for **2 min** at **11,000 x g** in order to remove wash buffer and dry the silica membrane completely.

+ 400 µl PW1

11,000 x g
1 min



+ 700 µl PW2

11,000 x g
1 min



+ 200 µl PW2

11,000 x g
2 min

7 Elute DNA

Place the NucleoSpin® Plant II Column into a new 1.5 ml microcentrifuge tube (not provided).

Pipette **50 µl Buffer PE (70°C)** onto the membrane. Incubate the NucleoSpin® Plant II Column for **5 min** at **70°C**. Centrifuge for **1 min** at **11,000 x g** to elute the DNA.

Repeat this step with another **50 µl Buffer PE (70°C)** and elute into the same tube.

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.



+ 50 µl PE
70°C
5 min

11,000 x g
1 min



+ 50 µl PE
70°C
5 min

11,000 x g
1 min

5.2 Support protocol for genomic DNA from fungi

Attention: Additional reagents and equipment necessary!

- Ethanol (96 – 100%)
 - Chloroform
 - Micro pistil
 - Siliconized glass beads or sea sand
-

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 into a 1.5 ml microcentrifuge tube (not provided). Add **150 mg siliconized glass beads** or **sea sand** and **200 µl Buffer PL1**. Homogenize sample using a micro pistil and vortex regularly. Add additional **100 µl Buffer PL1** and continue to homogenize the sample.

*Note: If the sample can not be handled easily because e.g. the sample material is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volume of **Buffer PC** (step 4) has to be increased proportionally.*

*Optional: If the sample is rich in RNA or protein, we recommend adding **10 µl RNase A and/or Proteinase K** (5 – 10 mg/ml stock solution, see ordering information), respectively, to the **PL1 lysis solution** in order to minimize contaminants.*

Incubate for **10 min** at **65°C**.

Add **100 µl chloroform**. Vortex for **10 s** and separate phases by centrifugation for **15 min** at **20,000 x g**. Pipette the top aqueous layer into a new 1.5 ml microcentrifuge tube (not provided).

For some fungi it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with section 5.1, step 3.

5.3 Support protocol for soil, compost, dung, and animal excrements

Attention: Additional equipment necessary!

- Bead mill (e.g., Pulverisette 0, Fritsch – Idar-Oberstein) or mortar and pestle
 - Sea sand (siliconized)
 - Extraction buffer: 2 M NaCl, 20 mM EDTA, 100 mM Tris/HCl, 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 microcentrifuge tube (not provided).

Proceed with section 5.1, step 3.

6 NucleoSpin® Plant II Midi protocol

Before starting the preparation:

- Check if Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 70°C.
- A centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,500 x g is required.

Note: The NucleoSpin® Plant II Midi kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

1 Homogenize sample

Homogenize up to 400 mg wet weight or up to 80 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.4).



Homogenize samples

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2a Cell lysis using Buffer PL1

Transfer the resulting powder to a new tube and add **1.7 ml Buffer PL1**. Vortex the mixture thoroughly.

+ 1.7 ml PL1

*Note: If the sample can not be resuspended easily because e.g. the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of **RNase A** (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.*

Add **25 µl RNase A** solution and mix sample thoroughly.



**+ 25 µl
RNase A**

Incubate the suspension for **10 min** at **65°C**.

**65°C
10 min**

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2b Cell lysis using Buffer PL2

Transfer the resulting powder to a new tube and add **1.5 ml Buffer PL2**. Vortex the mixture thoroughly.

+ 1.5 ml PL2

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of **RNase A**, **Buffer PL3** (step 2b), and **Buffer PC** (step 4) have to be increased proportionally.*

Add **25 µl RNase A** solution and mix sample thoroughly.

+ 25 µl
RNase A

Incubate the suspension for **10 min** at **65°C**.

65°C
10 min

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Add **200 µl Buffer PL3**, mix thoroughly and incubate for **5 min on ice** to precipitate SDS completely.

+ 200 µl PL3
0°C
5 min

Proceed with step 3.

3 Filtration / Clarification of crude lysate

Transfer the lysate to a **NucleoSpin® Filter L**. Centrifuge for **10 min** at **4,500 x g**, collect the clear flow-through and discard the NucleoSpin® Filter L.

If not all liquid has passed the filter, repeat the centrifugation step.

If a pellet is visible in the flow-through, transfer the clear supernatant to a new 15 ml microcentrifuge tube (not provided).



4,500 x g
10 min

Alternatively, centrifuge the crude lysate for 5 min at 4,500 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter L to remove solid particles completely.

4 Adjust DNA binding conditions

Add **2.3 ml Buffer PC** to the cleared lysate and mix immediately by vortexing for **30 s**.



+ 2.3 ml PC
Vortex 30 s

5 Bind DNA

Load sample on a **NucleoSpin® Plant II Midi Column**.

Centrifuge for **2 min** at **4,500 x g** and discard the flow-through.

The maximum loading capacity of the NucleoSpin® Plant II Midi Column is 5 ml. For higher sample volumes repeat the loading step.



Load sample



**4,500 x g
2 min**

6 Wash and dry silica membrane

1st wash

Add **1 ml Buffer PW1** to the NucleoSpin® Plant II Midi Column. Centrifuge for **2 min** at **4,500 x g** and discard flow-through.

2nd wash

Add **3 ml Buffer PW2** to the NucleoSpin® Plant II Midi Column. Centrifuge for **2 min** at **4,500 x g** and discard flow-through.

3rd wash

Add another **1 ml Buffer PW2** to the NucleoSpin® Plant II Midi Column. Centrifuge for **10 min** at **4,500 x g** in order to remove wash buffer and dry the silica membrane completely.



+ 1 ml PW1

**4,500 x g
2 min**

+ 3 ml PW2

**4,500 x g
2 min**



+ 1 ml PW2

**4,500 x g
10 min**

7 Elute DNA

Place the NucleoSpin® Plant II Midi Column into a new Collection Tube (15 ml)

Pipette **200 µl Buffer PE (70°C)** onto the membrane. Incubate the NucleoSpin® Plant II Midi Column for **5 min** at **70°C**. Centrifuge for **2 min** at **4,500 x g** to elute the DNA.

Repeat this step with another **200 µl Buffer PE (70°C)** and elute into the same tube.

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.



**+ 200 µl PE
70°C
5 min**

**4,500 x g
2 min**

**+ 200 µl PE
70°C
5 min**



**4,500 x g
2 min**

7 NucleoSpin® Plant II Maxi protocol

Before starting the preparation:

- Check if Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 70°C.
- A centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,500 x g is required.

Note: The NucleoSpin® Plant II Maxi kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

1 Homogenize sample

Homogenize up to 1500 mg wet weight or up to 300 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.4).



Homogenize samples

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2a Cell lysis using Buffer PL1

Transfer the resulting powder to a new tube and add **6 ml Buffer PL1**. Vortex the mixture thoroughly.

+ 6 ml PL1

Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of **RNase A** (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.

Add **100 µl RNase A** solution and mix sample thoroughly.



**+ 100 µl
RNase A**

Incubate the suspension for **10 min** at **65°C**.

**65°C
10 min**

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2b Cell lysis using Buffer PL2

Transfer the resulting powder to a new tube and add **5.3 ml Buffer PL2**. Vortex the mixture thoroughly.

+ 5.3 ml PL2

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of **RNase A**, **Buffer PL3** (step 2b), and **Buffer PC** (step 4) have to be increased proportionally.*

Add **100 µl RNase A** solution and mix sample thoroughly.

+ 100 µl
RNase A

Incubate the suspension for **10 min** at **65°C**.

65°C
10 min

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Add **700 µl Buffer PL3**, mix thoroughly and incubate for **5 min on ice** to precipitate SDS completely.

+ 700 µl PL3
0°C
5 min

Proceed with step 3.

3 Filtration / Clarification of crude lysate

Transfer the lysate to a **NucleoSpin® Filter XL**. Centrifuge for **10 min** at **4,500 x g**, collect the clear flow-through and discard the NucleoSpin® Filter XL.

If not all liquid has passed the filter, repeat the centrifugation step.

If a pellet is visible in the flow-through, transfer the clear supernatant to a new 50 ml microcentrifuge tube (not provided).

Alternatively, centrifuge the crude lysate for 5 min at 4,500 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter XL to remove solid particles completely.



4,500 x g
10 min

4 Adjust DNA binding conditions

Add **8 ml Buffer PC** to the cleared lysate and mix immediately by vortexing for **30 s**.

+ 8 ml PC
Vortex 30 s



5 Bind DNA

Load sample on a **NucleoSpin® Plant II Maxi Column**

Centrifuge for **2 min** at **4,500 x g** and discard the flow-through.

The maximum loading capacity of the NucleoSpin® Plant II Maxi Column is 15 ml. For higher sample volumes repeat the loading step.



Load sample



**4,500 x g
2 min**

6 Wash and dry silica membrane

1st wash

Add **4 ml Buffer PW1** to the NucleoSpin® Plant II Maxi Column. Centrifuge for **2 min** at **4,500 x g** and discard flow-through.

+ 4 ml PW1

**4,500 x g
2 min**

2nd wash

Add **10 ml Buffer PW2** to the NucleoSpin® Plant II Maxi Column. Centrifuge for **2 min** at **4,500 x g** and discard flow-through.

+ 10 ml PW2

**4,500 x g
2 min**

3rd wash

Add another **2 ml Buffer PW2** to the NucleoSpin® Plant II Maxi Column. Centrifuge for **10 min** at **4,500 x g** in order to remove wash buffer and dry the silica membrane completely.

+ 2 ml PW2

**4,500 x g
10 min**

7 Elute DNA

Place the NucleoSpin® Plant II Maxi Column into a new Collection Tube (50 ml)

Pipette **1000 µl Buffer PE (70°C)** onto the membrane. Incubate the NucleoSpin® Plant II Maxi Column for **5 min** at **70°C**. Centrifuge for **2 min** at **4,500 x g** to elute the DNA.

**+ 1000 µl PE
70°C
5 min**

**4,500 x g
2 min**

Repeat this step with another **1000 µl Buffer PE (70°C)** and elute into the same tube.

**+ 1000 µl PE
70°C
5 min**

**4,500 x g
2 min**

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.

8 Appendix

8.1 Troubleshooting

| Problem | Possible cause and suggestions |
|------------------|--|
| DNA yield is low | <p><i>Homogenization of plant material was not sufficient</i></p> <ul data-bbox="337 379 981 552" style="list-style-type: none"> • For most species we recommend grinding with steel beads or mortar and pestle (see section 2.4). For disruption of the cell wall it is important to homogenize the plant material thoroughly until the sample is ground to a fine powder. • Instead of freezing in liquid nitrogen the sample can also be lyophilized and easily ground at room temperature. |
| | <p><i>Suboptimal lysis buffer was used</i></p> <ul data-bbox="337 627 981 730" style="list-style-type: none"> • 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. |
| | <p><i>Suboptimal lysis buffer volume was used</i></p> <ul data-bbox="337 805 981 909" style="list-style-type: none"> • Cell lysis might be insufficient and too much DNA might get lost during lysate clarification if e.g. dry material soaks up too much lysis buffer. Use more lysis buffer and increase the volume of Binding Buffer PC proportionally. |
| | <p><i>Suboptimal binding buffer volume was used</i></p> <ul data-bbox="337 984 981 1034" style="list-style-type: none"> • Increase Binding Buffer PC proportionally if more lysis buffer was used. |
| | <p><i>Extraction of DNA from plant material during lysis was insufficient</i></p> <ul data-bbox="337 1137 936 1158" style="list-style-type: none"> • Increase incubation time in lysis buffer (up to overnight). |
| | <p><i>Suboptimal Elution</i></p> <ul data-bbox="337 1233 981 1434" style="list-style-type: none"> • The DNA can either be eluted in higher volumes or by repeating the elution step up to three times. Incubate NucleoSpin® Plant II Column with elution buffer at 70°C for at least 5 minutes. • Also check the pH of the elution buffer, which should be in the range of pH 8.0 – 8.5. To ensure correct pH, use supplied Elution Buffer PE (5 mM Tris/HCl, pH 8.5). |

Problem

Possible cause and suggestions

NucleoSpin®
Filter or
NucleoSpin®
Plant II Column
is clogged

Sample was too viscous due to too much sample material or material carry-over.

- Centrifuge large amounts of sample material before loading it onto the NucleoSpin® Filter or Filter L/XL.
- Make sure the cleared lysate is absolutely free of resuspended matter before loading it onto the NucleoSpin® Plant II or Plant II Midi/Maxi Column.
- Increase centrifugation speed.
- Use more Lysis Buffer PL1 or PL2.

DNA is
degraded

Sample was contaminated with DNase

- Preheat elution buffer to 70°C for 5 min to eliminate 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 contaminants like phenolic compounds or secondary metabolites

- Use optional washing step with Wash Buffer PW1 and repeat this step if necessary.

Elution buffer contains EDTA

- EDTA may disturb subsequent reactions. Use water or the supplied Elution Buffer PE (5 mM Tris/HCl, pH 8.5) for elution.

Salt or ethanol carry-over

- Make sure the last two wash steps were done with Wash Buffer PW2 and the membrane was dried according to the protocol.

8.2 Ordering information

| Product | Cat. No. | Pack of |
|--|---------------------|-----------------|
| NucleoSpin® Plant II | 740770.10/.50/.250 | 10/50/250 preps |
| NucleoSpin® Plant II Midi | 740771.20 | 20 preps |
| NucleoSpin® Plant II Maxi | 740772.10 | 10 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 | 740505.50 740505 | 50 mg 100 mg |
| Proteinase K | 740506 | 100 mg |
| NucleoSpin® Filters (for filtration of cell homogenates) | 740606 | 50 |
| Collection Tubes (2 ml) | 740600 | 1000 |

8.3 Product use restriction/warranty

NucleoSpin® Plant II/Midi/Maxi kit 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 II/Midi/Maxi** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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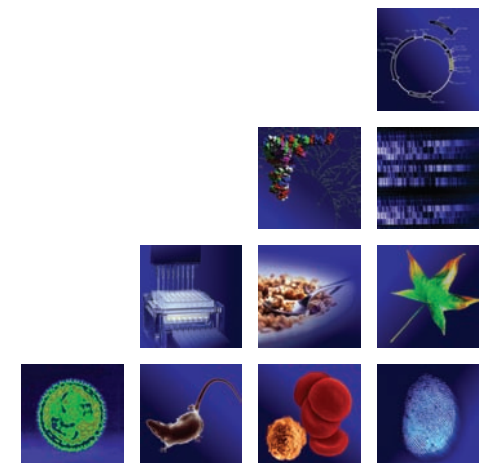
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