HANDBOOK

Genomic DNA Purification from Plant

☐	**DNASure® Plant Mini Kit**	NP-79105	50 Preps
☐	**DNASure® Plant Mini Kit**	NP-79107	250 Preps
☐	**DNASure® Plant Midi Kit**	NP-78153	20 Preps
☐	**DNASure® Plant Maxi Kit**	NP-78164	10 Preps

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COMPONENTS

Kit contents

DNASure Plant

Cat. No.	NP-79105	NP-79107
Number of Preps	50 preps	250 preps
Lysis BufferLB1	25 ml	125 ml
Lysis Buffer LB2	20 ml	100 ml
Precipitation Buffer PPB3	5 ml	25 ml
Binding Buffer BBC	30 ml	125 ml
Wash Buffer WB1	30 ml	125 ml
Wash Buffer WB2 (Concentrate)*	25 ml	50 ml
Elution Buffer PE	15 ml	30 ml
Rnase A (lyophilized)*	6 mg	2 x 15 mg
DNASure® Shredder Mini Column	50	250
DNASure® Plant Columns	50	250
Collection Tubes (2 ml)	100	500
Handbook	1	1

	DNASure Plant Midi	DNASure Plant Maxi
Cat. No.	NP-78153	NP-78164
Number of Preps	20 preps	10 preps
Lysis Buffer LB1	2 x 25 ml	75 ml
Lysis Buffer LB2	2 x 20 ml	60 ml
Precipitation Buffer PPB3	5 ml	15 ml
Binding Buffer BBC	2 x 30 ml	125 ml
Wash Buffer WB1	30 ml	50 ml
Wash Buffer WB2 (Concentrate)*	25 ml	50 ml
Elution Buffer PE	15 ml	30 ml
Rnase A (lyophilized)*	6 mg	10 mg
DNASure® Shredders Midi/Maxi Columns (plus Collection Tubes)	20	10
DNASure® Plant Midi / Maxi Columns (plus Collection Tubes)	20	10
Collection Tubes (15 ml / 50 ml)	20	10
Handbook	1	1

* Please see "Preparation of Reagents"

Reagents, consumables, and equipment not supplied with the kit

- 96 – 100 % ethanol
- 1.5 ml microcentrifuge tubes (DNASure® Plant) or 15 / 50 ml tubes (DNASure® Plant Midi / Maxi)
- Disposable tips
- Pipettes
- Centrifuge with swing-out rotors capable of reaching 4,500 x g for 15 ml / 50 ml tubes (DNASure® Plant Mini, Midi/Maxi) Dry Bath or water bath Tissue Ruptor or Mortar/Pestle.

SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs).

Buffers BBC and WB1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.
The following risk and safety phrases apply to components of the DNASure® Plant Kit:

Binding Buffer BBC
Contain Guanidine hydrochloride + ethanol <40%: R&S Phrases: R10-22-36/38, S7-16

Wash Buffer WB1
Contain Guanidine hydrochloride + Contain Guanidine hydrochloride + isoprapanol <25%: R&S Phrases: R10-22-36/38, S7-16-25

RNase A
Contain lyophilized RNase A: R&S Phrases: R42/43, S22-24

R10: Flammable, R22: Harmful if swallowed, R36/38: Irritating to eyes and skin, May cause sensitization by inhalation and skin contact, R42/43: May cause sensitization by inhalation and skin contact, S7: Keep container tightly closed, S16: Keep away from sources of ignition - No smoking, S22: Do not breathe dust, S24: Avoid contact with skin, S25: Avoid contact with eyes

INTRODUCTION

Principle and Procedure

Plant material is first mechanically disrupted and then lysed by addition of two Lysis Buffers LB1 and LB2 and incubation. RNase A in the lysis buffer digests the RNA in the sample. After lysis, proteins and polysaccharides are precipitated. Crude lysate is cleared by centrifugation and/or DNASure® Shredder columns provided with the kits in order to remove polysaccharides, contaminations, and residual cellular debris

The flow-through is mixed with Binding Buffer BBC 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 WB1 and WB2. DNA binds to the membrane while contaminants such as proteins and polysaccharides are efficiently removed by two wash steps. Pure DNA is eluted in a small volume of low-salt buffer or water.

Specifications of DNASure Plant Kits

DNASure® Plant kits are specifically designed for the genomic DNA isolation from plant tissue and other biological samples like soil using two lysis buffer systems based on the conventional CTAB and SDS methods. DNASure® Shredder columns 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 BBC and the optional chaotropic Wash Buffer WB1 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.

Starting material and typical yield

- 1-30 µg of DNA can be extracted from upto 100 mg wet weight or 20 mg dry weight of sample in 100µl elution volume using DNASure® Plant Mini Kit.
- 10-100 µg of DNA can be extracted from 400 mg wet weight or 80 mg dry weight of sample in 400µl elution volume using DNASure® Plant Midi Kit.
- 50-300 µg of DNA can be extracted from 1500mg wet weight of 300 dry weight of sample in 2000 µl of elution volume using DNASure® Plant Maxi Kit.

Storage

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

Homogenization of plant samples

Plant tissue can be homogenized to powdered under liquid nitrogen using mortar pestle. Commercial homogenizers (rotor-stator homogenizer) or bead mills using steel or glass beads. Can also be used. However, it is recommend to grind samples using pre cooled 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 DNASure® Shredder columns.

Lysis

Increasing the amount of starting material

The standard protocols of DNASure® Plant / Midi / Maxi kits allow processing of 10 – 1500 mg of plant material. The typical yield varies between 1 – 300 µg of high quality DNA depending upon size and ploidy of the genome. For example 100 mg fresh wheat with a hexaploid genome (1.7 x 10^{10} bp) contains 30 µg DNA, whereas the same amount of Arabidopsis with a smaller diploid genome (1.9 x 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.

Choosing the optimal lysis buffer system

We offer two different lysis buffers for the optimal processing, high yields, and an excellent DNA quality with most common plant species as they are heterogeneous and contain varying amounts of polyphenols, acidic components, or polysaccharides which can lead to suboptimal DNA extraction or performance in downstream applications. The standard protocol uses Lysis Buffer LB1, which is based on the conventional CTAB buffer procedure. Additionally, the SDS based Lysis Buffer LB2 is provided which requires subsequent protein precipitation by potassium acetate (Precipitation Buffer PPB3). For some plant species Lysis Buffers LB1 and LB2 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.

Preparation and storage of reagents

Precautions:

- Buffers LB1, LB2, BBC, and WB1 contain guanidinium hydrochloride and/or detergents like CTAB or SDS
- All kit components can be stored at room temperature (18 – 25 °C) and are stable up to one year.

Prepare Lysis Buffer LB2: Check for precipitated SDS especially after storage at temperatures below -20°C, incubate the bottle for several minutes at 30 – 40 °C and mix well until the precipitate dissolves completely.

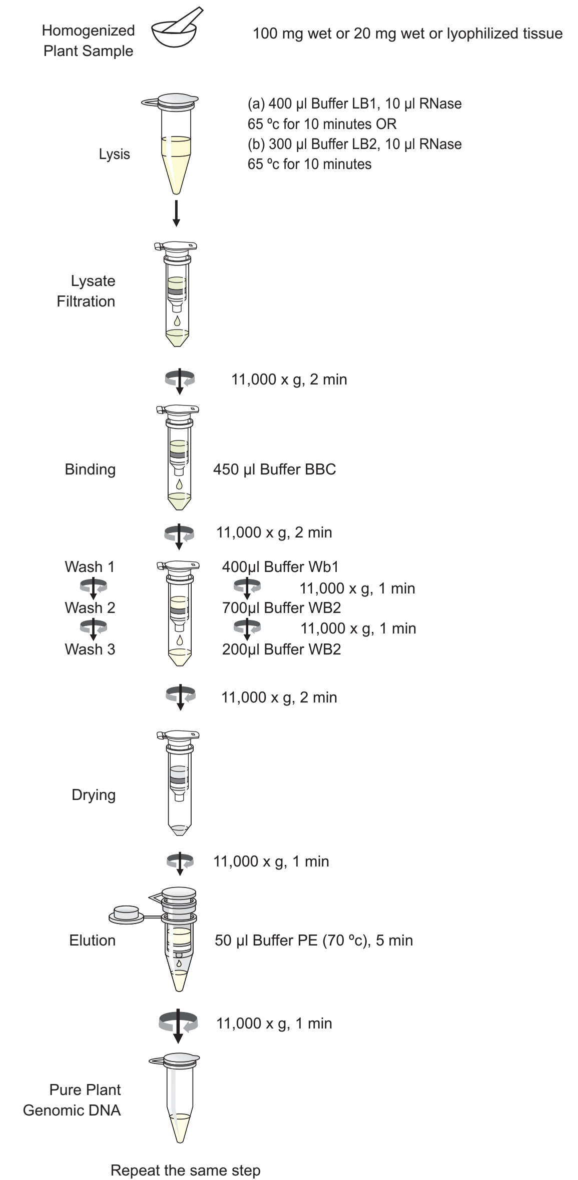
Reconstitute Wash Buffer WB2:

Add 100ml of ethanol (96-100%) to Buffer WB2 concentrate (Kits NP-79105 and NP-78153), similarly, add 200ml of ethanol (96-100%) to Buffer WB2 concentrate (Kits NP-79107 and NP-78164). Mark the bottle as "Ethanol Added". Buffer WB2 is stable at room temperature (18-25°C) for at least one year.

RNase A

Add 600ul of RNase Free water to 6mg of lyophilized RNase A (Kits NP-79105 and NP-78153), 1.5ml of RNase Free water to 15mg of RNase A (Kit NP-79107) and 1.1ml of RNase Free water to 10mg of RNase A (Kit NP-78164). Store RNase A solution at 4°C for upto 3 months. For longer storage upto one year, dispense RNase A solution in small aliquots and store at -20°C.

Genomic DNA Purification from Plant



Protocol for purification of Genomic DNA from plant

Genomic DNA purification with DNASure Plant Mini Kit

Things to do Before starting:

- Check if Wash Buffer WB2 and RNase A were prepared as per instruction.
- Preheat Elution Buffer PE to 70°C.
  Use appropriate Lysis buffer for optimal results with most common plant species.

Procedure

1 **Homogenize up to 100 mg wet weight (or up to 20 mg dry weight or lyophilized) plant material (see homogenization of plant samples).**

Proceed with Buffer LB1 (step 2 a) or alternatively with Buffer LB2 (step 2 b).

2a **Transfer the resulting powder (from step 1) to a fresh tube and add 400 µl Buffer LB1. Vortex the mixture thoroughly.** Note: If required additional Buffer LB1 can be added. ***Add 10* µl RNase A solution and mix sample thoroughly. Incubate the suspension at 65 C for 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 **Optional: Transfer the resulting powder (from step 1) to a fresh tube and add 300 µl Buffer LB2. 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 LB2 can be added. **Add 10 µl RNase A solution and mix sample by pipetting up and down. Incubate the suspension at 65 C for 10 min.** Note: For certain plant material it might be advantageous to increase the incubation time to 30 – 60 min. Add 75 µl Buffer PPB3, mix thoroughly and incubate for 5 minutes on ice to precipitate SDS completely.
Proceed with step 3.

3. **Place a DNASure Shredder into a fresh 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 DNASure Shredder Mini Column.** Repeat the centrifugation if not all liquid has passed the filter,. If a pellet is visible in the flow-through, transfer the clear supernatant to a fresh 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 fresh tube or pass the precleared supernatant through the DNASure® Shredder Mini Column to remove solid particles completely.

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

5 **Place a DNASure Plant Column into a fresh 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 flowthrough.** The maximum loading capacity of the DNASure® Plant Column is 700 µl. For higher sample volumes repeat the loading step.

6 **Add 400 µl Buffer WB1 to the DNASure Plant Column. Centrifuge for 1 min at 11,000 x g and discard flow-through.**

7 **Add 700 µl Buffer WB2 to the DNASure Plant Column. Centrifuge for 1 min at 11,000 x g and discard flow-through.**

8 **Add another 200 µl Buffer WB2 to the DNASure Plant Column. Centrifuge for 2 min at 11,000 x g in order to remove wash buffer and dry the silica membrane completely.**

9a **Place the DNASure Plant Column into a fresh 1.5 ml microcentrifuge tube (not provided). Pipette 50 µl Buffer PE (70 C) onto the membrane. Incubate the DNASure Plant Column for 5 min at 70 C. Centrifuge for 1 min at 11,000 x g to elute the D*NA.***

9b **Repeat this step with another 50 µl Buffer PE (70 C) and elute into the same tube.**
Note: In order to get maximum yield use 100 µl of pre-heated elution buffer (at 70°C for 5 minutes) in steps 9a and 9b. To achieve high concentration (75%) use 25 µl of pre-heated elution buffer in steps 9a and 9b.

Supplementary Protocol for purification of genomic DNA from fungi

Equipment required but not provided with the kit

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

Procedure

1 **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 **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 LB1. Homogenize sample using a micro pistil and vortex regularly. Add additional 100 µl Buffer LB1 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 LB1 can be added. Note that the volume of Buffer BBC (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 LB1 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 fresh 1.5 ml microcentrifuge tube (not provided).
Note: For some fungi it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3 of standard protocol

Supplementary Protocol for purification of Genomic DNA from soil, compost and animal excrements

Equipment required but not provided with the kit

- Bead mill or mortar and pestle
- Sea sand (siliconized)
- Extraction buffer: pH 8.0

Procedure

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

3 **Transfer the homogenized sample into a centrifuge tube and centrifuge for 10 min at 5,000 x g. Pipette 300 µl of the clear supernatant into a fresh 1.5 ml microcentrifuge tube (not provided).**
Proceed with step 3 of standard protocol

Protocol for purification of Genomic DNA from Plant

Genomic DNA purification with DNASure Plant Midi Kit

Things to do Before starting:

- Check if Wash Buffer WB2 and RNase A were prepared as per instructions.
- 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 DNASure® Plant 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.

Procedure

1 **Homogenize up to 400 mg wet weight or up to 80 mg dry weight (lyophilized) plant material (see homogenization of plant sample).**

Proceed with cell lysis using Buffer LB1 (step 2 a) or alternatively with Buffer LB2 (step 2b).

2a **Transfer the resulting powder (from step 1) to a fresh tube and add 1.7 ml Buffer LB1. Vortex the mixture thoroughly.** Note: If the sample can not be resuspended easily because e.g. the plant powder is soaking up too much buffer, additional Buffer LB1 can be added. **Add 25 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.

Proceed with step 3.

2b **Optional: Transfer the resulting powder (from step 1) to a fresh tube and add 1.5 ml Buffer LB2. 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 LB2 can be added. **Add 25 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 200 µl Buffer PPB3, mix thoroughly and incubate for 5 min on ice to precipitate SDS completely.**

Proceed with step 3.

3 **Transfer the lysate to a DNASure Shredder Midi Column. Centrifuge for 10 min at 4,500 x g, collect the clear flow-through and discard the DNASure Shredder Midi Column. 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 fresh 15 ml microcentrifuge tube (not provided).**

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

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

5 **Load sample on a DNASure Plant Midi Column. Centrifuge for 2 min at 4,500 x g and discard the flowthrough. The maximum loading capacity of the DNASure Plant Midi Column is 5 ml. For higher sample volumes repeat the loading step.**

6 **Add 1 ml Buffer WB1 to the DNASure Plant Midi Column. Centrifuge for 2 min at 4,500 x g and discard flow-through.**

7 **Add 3 ml Buffer WB2 to the DNASure Plant Midi Column. Centrifuge for 2 min at 4,500 x g and discard flow-through.**

8 **Add 1 ml Buffer WB2 to the DNASure Plant Midi Column. Centrifuge for 10 min at 4,500 x g in order to remove wash buffer and dry the silica membrane completely.**

9a **Place the DNASure Plant Midi Column into a fresh Collection Tube (15 ml) Pipette 200 µl Buffer PE (70 C) onto the membrane. Incubate the DNASure Plant Midi Column for 5 min at 70 C. Centrifuge for 2 min at 4,500 x g to elute the DNA.**

9b **Repeat this step with another 200 µl Buffer PE (70 C) and elute into the same tube.**

Note: In order to get maximum yield use 100 µl of pre-heated elution buffer (at 70°C for 5 minutes) in steps 9a and 9b. To achieve high concentration (75%) use 25 µl of pre-heated elution buffer in steps 9a and 9b.

Protocol for purification of Genomic DNA from Plant

Genomic DNA purification with DNASure Plant Maxi Kit

Things to do Before starting:

- Check if Wash Buffer WB2 and RNase A were prepared as per instructions
- 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 DNASure® Plant 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 Homogenization

Procedure

1 **Homogenize up to 1500 mg wet weight or up to 300 mg dry weight (lyophilized) plant material (see homogenization of plant samples).**

Proceed with cell lysis using Buffer LB1 (step 2 a) or alternatively with Buffer LB2 (step 2b).

2a **Transfer the resulting powder to a fresh tube and add 6 ml. Buffer LB1. 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 LB1 can be added. Note that the volumes of RNase A (step 2 a) and Buffer BBC (step 4) have to be increased proportionally.* **Add 100 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.

Proceed with step 3.

2b **Transfer the resulting powder to a fresh tube and add 5.3 ml Buffer LB2. 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 LB2 can be added. Note that the volumes of RNase A, Buffer PPB3 (step 2 b), and Buffer BBC (step 4) have to be increased proportionally. ***A*dd 100 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 700 µl Buffer PPB3, mix thoroughly and incubate for 5 min on ice to precipitate SDS completely.**

Proceed with step 3.

3 **Transfer the lysate to a DNASure Shredder Midi Column. Centrifuge for 10 min at 4,500 x g, collect the clear flow-through and discard the DNASure Shredder Midi Column. 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 fresh 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 fresh tube or pass the precleared supernatant through the DNASure® Shredder XL to remove solid particles completely.

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

5 **Load sample on a DNASure Plant Maxi Column Centrifuge for 2 min at 4,500 x g and discard the flowthrough.** The maximum loading capacity of the DNASure® Plant Maxi Column is 15 ml. For higher sample volumes repeat the loading step.

6 **Add 4 ml Buffer WB1 to the DNASure Plant Maxi Column. Centrifuge for 2 min at 4,500 x g and discard flow-through.**

7 **Add 10 ml Buffer WB2 to the DNASure Plant Maxi Column. Centrifuge for 2 min at 4,500 x g and discard flow-through.**

8 **Add another 2 ml Buffer WB2 to the DNASure Plant Maxi Column. Centrifuge for 10 min at 4,500 x g in order to remove wash buffer and dry the silica membrane completely.**

9a **Place the DNASure Plant Maxi Column into a fresh Collection Tube (50 ml). Pipette 1000 µl Buffer PE (70 C) onto the membrane. Incubate the DNASure Plant Maxi Column for 5 min at 70 C. Centrifuge for 2 min at 4,500 x g to elute the DNA.**

9b **Repeat this step with another 1000 µl Buffer PE (70 C) and elute into the same tube.**

Note: In order to get maximum yield use 100 µl of pre-heated elution buffer (at 70°C for 5 minutes) in steps 9a and 9b. To achieve high concentration (75%) use 25 µl of pre-heated elution buffer in steps 9a and 9b.

TROUBLESHOOTING GUIDE

DNA yield is low

Possible cause
- Homogenization of plant material was not sufficient

Suggestion(s)
- For most species we recommend grinding with steel beads or mortar and pestle. 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.

Possible cause
- Suboptimal lysis buffer was used

Suggestion(s)
- Lysis efficiencies of Buffer LB1 (CTAB) and Buffer LB2 (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.

Possible cause
- Suboptimal lysis buffer volume was used

Suggestion(s)
- 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 BBC proportionally.

Possible cause
- Suboptimal binding buffer volume was used

Suggestion(s)
- Increase Binding Buffer BBC proportionally if more lysis buffer was used.

Possible cause
- Extraction of DNA from plant material during lysis was insufficient

Suggestion(s)
- Increase incubation time in lysis buffer (up to overnight).

Possible cause
- Suboptimal Elution

Suggestion(s)
- The DNA can either be eluted in higher volumes or by repeating the elution step up to three times. Incubate DNASure® Plant 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).

DNASure Shredder or DNASure Plant Column is clogged

Possible cause
- Sample was too viscous due to too excess of starting material.

Suggestion(s)
- Centrifuge large amounts of sample material before loading it onto the DNASure® Shredder or ShredderMidi/Maxi.
- Make sure the cleared lysate is absolutely free of resuspended matter before loading it onto the DNASure® Plant or Plant Midi / Maxi Column.
- Increase centrifugation speed.
- Use more Lysis Buffer LB1 or LB2.

DNA is Degrated

Possible cause
- Sample was contaminated with DNase

Suggestion(s)
- Preheat elution buffer to 70 °C for 5 min to eliminate DNase contamination. This precaution is not necessary for buffers supplied by , which are delivered free of RNase and Dnase.

Possible cause
- Centrifugation speed was too high

Suggestion(s)
- Centrifuge at a maximum speed of 11,000 x g. Higher velocities may lead to shearing of the DNA.

DNA quality is low

Possible cause

n **Sample contains contaminants like phenolic compounds or secondary metabolites**

Suggestion(s)
- Use optional washing step with Wash Buffer WB1 and repeat this step if necessary.

Possible cause
- Elution buffer contains EDTA

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

Possible cause
- Salt or ethanol carry-over

Suggestion(s)
- Make sure the last two wash steps were done with Wash Buffer WB2 and the membrane was dried according to the protocol.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
DNASure® Tissue Mini Kit	50 preps	NP-61305
DNASure® Plant Mini Kit	50 preps	NP-79105
DNASure® Plant Mini Kit	250 preps	NP-79107
DNASure® Plant Midi Kit	20 preps	NP-78153
DNASure® Plant Maxi Kit	10 preps	NP-78164
DNASure® Blood Mini Kit	50 preps	NP-61105
DNASure® Blood Mini Kit	250 preps	NP-61107
DNASure® Blood Midi Kit	20 preps	NP-61184
DNASure® Blood Maxi Kit	10 preps	NP-61193
DNASure® Blood FastPure Kit	50 preps	NP-62205
DNASure® Blood FastPure Kit	250 preps	NP-62207
SureSpin® Plasmid Mini Kit	50 preps	NP-37105
SureSpin® Plasmid Mini Kit	250 preps	NP-37107
SureSpin® Plasmid FastPrep Kit	50 preps	NP-47105
SureSpin® Plasmid FastPrep Kit	250 preps	NP-47107
SureSpin® Buffer Set*	1	37107-BS
SurePrep® Plasmid Mini Kit	20 preps	NP-15123
SurePrep® Plasmid Mini Kit	100 preps	NP-15125
SurePrep® Plasmid Midi Kit	20 preps	NP-15143
SurePrep® Plasmid Midi Kit	100 preps	NP-15145
SurePrep® Plasmid Maxi Kit	10 preps	NP-15161
SurePrep® Plasmid Maxi Kit	25 preps	NP-15162
SurePrep® Plasmid Mega Kit	5 preps	NP-15183
SurePrep® Plasmid Giga Kit	5 preps	NP-15191

***SureSpin Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

Product Warranty

DNASure® Plant Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the DNASure® Plant Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of DNASure® Plant Kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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