DNeasy® Plant Mini Kit and DNeasy Plant Maxi Kit Handbook

For DNA isolation from plant tissue

August 2000

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Kit Contents

	DNeasy® Plant Mini Kit			DNeasy Plant Maxi Kits	
Catalog No.	**69103**	**69104**	**69106**	**68161**	**68163**
Number of preparations	**20**	**50**	**250**	**6**	**24**
DNeasy spin columns (colorless)	20	50	250	6	24
QIAshredder™ spin columns (lilac)	20	50	250	6	24
Collection tubes (2 ml)	20	50	250	–	–
Collection tubes (50 ml)	–	–	–	6	24
Buffer AP1	15 ml	40 ml	200 ml	40 ml	140 ml
Buffer AP2	10 ml	18 ml	90 ml	18 ml	50 ml
Buffer AP3/E* (concentrate)†	12 ml	30 ml	125 ml	25 ml	2 x 50 ml
Buffer AW (concentrate)†	7 ml	17 ml	81 ml	26 ml	2 x 54 ml
Buffer AE	12 ml	2 x 12 ml	2 x 60 ml	15 ml	60 ml
RNase A (100 mg/ml)	110 µl	220 µl	5 x 220 µl	110 µl	440 µl
Handbook	1	1	1	1	1

* *Contains chaotropic salt which is an irritant. Take appropriate safety measures. Buffer AP3/E is not compatible with disinfecting agents which contain bleach.*

† *Buffers AP3/E and AW are supplied as concentrates. Add ethanol (96–100%) according to the bottle label before use to obtain a working solution.*

Storage Conditions

All components of DNeasy Plant Kits including RNase A stock solution should be stored dry, at room temperature (15–25°C) and are stable for 1 year under these conditions. For storage in tropical climates we suggest keeping the RNase A at 2–8°C.

Kit Specifications

DNeasy Plant Kit Specifications	Mini	Maxi
Maximum amount of starting material*	100 mg wet weight	1 g wet weight
	20 mg dry weight	0.2 g dry weight
DNeasy mini spin column specifications		
DNA binding capacity[†]	50 µg	500 µg
Maximum loading volume	700 µl	15 ml
Minimum elution volume	50 µl	500 µl
QIAshredder specifications		
Maximum loading volume	700 µl	15 ml

** Exceeding the recommended amount of starting material will reduce yield and purity.*

** Typically, the binding capacity will not be reached since the DNA content of the recommended amounts of starting material will not exceed 30 µg (mini) or 300 µg (maxi).*

Product Use Limitations

DNeasy Plant Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. 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, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor.

Safety Information

CAUTION:
DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The sample-preparation waste contains guanidine hydrochloride from Buffer AP3/E, which can form highly reactive compounds when combined with bleach.

In case 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.

Below is listed European Community risk and safety phrases for the components of the DNeasy Plant Mini and Maxi Kits to which they apply.

Buffer AP3/E

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22, R36/38, S2, S13, S26, S36, S46

Buffer AP2

Contains acetic acid: irritant. Risk and safety phrases: R36/38, S13, S26, S36, S46

RNase A

Sensitizer. Risk and safety phrases: R42/43, S23, S24, S26, S36/37

* *R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R42: May cause sensitization by inhalation; R42/43: May cause sensitization by inhalation and skin contact; S2: Keep out of children's reach; S13: Keep away from food, drink, and animal feed; S23: Do not breathe vapor; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S46: If swallowed, seek medical advice immediately, and show this notice to your doctor.*

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding DNeasy Plant Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors listed on the last page.

Reagents and Equipment to Be Supplied by User

- Equipment for disruption
- Ethanol (96–100%)
- Liquid nitrogen

For the DNeasy Plant Mini Kit:

- Microcentrifuge tubes (1.5 ml)
- Microcentrifuge with rotor for 2 ml tubes

For the DNeasy Plant Maxi Kit:

- Laboratory centrifuge (capable of 3000–5000 x *g*) equipped with a swing-out rotor.

  All centrifugation steps are carried out in a conventional laboratory centrifuge e.g., Beckman CS-6KR, Heraeus Varifuge® 3.0R, or Sigma 6K10 using a swinging bucket rotor. DNeasy maxi spin columns and QIAshredder maxi spin columns fit into the 50 ml centrifuge tubes provided. These tubes are compatible with almost all laboratory centrifuges and rotors. In the unlikely event that these tubes do not fit your rotor, the spin columns can also be used with any other commercially available 50 ml polypropylene or glass tubes.
- 15 ml and 50 ml centrifugation tubes; the use of disposable polypropylene tubes is recommended. Tubes used for the lysis step should be capable of resisting the *g* forces involved in centrifugation and also liquid nitrogen.

** Liquid nitrogen can cause severe burns on exposed skin. Take appropriate safety measures.*

Introduction

DNeasy Plant Kits provide a fast and simple way to isolate DNA from plant and fungal tissue. Up to 100 mg of tissue can be processed with the DNeasy Plant Mini Kits or up to 1 g of tissue can be processed with the DNeasy Plant Maxi Kits. The simple DNeasy spin column procedure yields pure total DNA (genomic, mitochondrial, chloroplast) for reliable PCR and Southern blotting in less than 1 hour (DNeasy Plant Mini Kit) or 2 hours (DNeasy Plant Maxi Kit). Purification requires no phenol or chloroform extraction or alcohol precipitation, and involves minimal handling. This makes DNeasy spin columns ideal for simultaneous processing of multiple samples. DNA is eluted in low-salt buffer or water, ready for use in downstream applications. DNA purified with DNeasy spin columns ranges in size up to 40 kb, with fragments of approximately 20–25 kb predominating. DNA of this length denatures completely in PCR and shows the highest amplification efficiency.

The DNeasy membrane ensures complete removal of all inhibitors of PCR and other enzymatic reactions. DNA purified with the DNeasy Plant Mini Kit is ideal for use in demanding downstream applications including:

- PCR
- RAPD analysis
- AFLP analysis
- RFLP analysis
- Blotting
- Microsatellite analysis
- Real-time PCR

DNeasy Plant Principle and Procedure

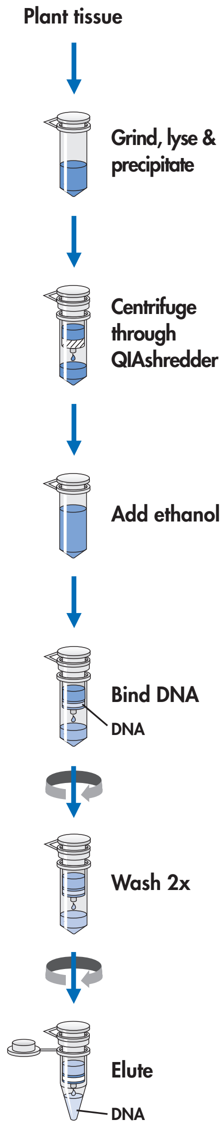
In the DNeasy Plant procedure (see flowchart, page 10), plant material is first mechanically disrupted and then lysed by addition of lysis buffer and incubation at 65°C. RNase in the lysis buffer digests the RNA in the sample. After lysis, proteins and polysaccharides are salt precipitated. Cell debris and precipitates are removed in a single step by a brief spin through QIAshredder™, a unique filtration and homogenization unit. The cleared lysate is transferred to a new tube and binding buffer and ethanol are added to promote binding of the DNA to the DNeasy membrane. The sample is then applied to a DNeasy spin column and spun briefly in a microcentrifuge. 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. DNeasy purified DNA has A_{260} / A_{280} ratios of 1.7–1.9 and absorbance scans show a symmetric peak at 260 nm confirming high purity.

The DNeasy membrane combines the binding properties of a silica-gel–based membrane with microspin technology. DNA adsorbs to the DNeasy membrane in the presence of high concentrations of chaotropic salt, which remove water from hydrated molecules in solution. Buffer conditions in the DNeasy Plant procedure are designed to allow specific adsorption of DNA to the silica-gel membrane and optimal removal of carbohydrates, polyphenolics, and other plant metabolites.

High-Throughput Sample Processing

For high-throughput needs, QIAGEN offers the DNeasy 96 Plant Kit — DNeasy Plant technology in a convenient, 96-well format for DNA isolation from plant tissues. For more information please contact your local Technical Services Department or distributor (listed on the last page of this handbook).

DNeasy Plant Procedure



The DNeasy Plant procedure

Technical Information

Collection and storage of starting material

After harvesting, plant tissue should be frozen in liquid nitrogen. It can then be stored at –80°C for later processing. Ground tissue powder can also be stored at –80°C. Alternatively, tissue can be dried after harvesting to allow storage at room temperature. To ensure DNA quality, samples should be completely dried within 24 hours of collection.

If possible it is preferable to collect young materials (e.g., leaves, needles) since they contain more cells per weight and therefore result in higher yields. In addition, young leaves/needles contain less polysaccharides and polyphenolics and are therefore easier to handle.

When working with fungi, harvest mycelium directly from a culture dish or from liquid culture. For liquid culture, first pellet cells by centrifugation. Remove the supernatant completely before disruption and lysis. Fresh, frozen, or freeze dried fungal material can be used.

Sample size

The DNeasy Plant Mini and Maxi procedures are optimized for maxima of 100 or 1000 mg of wet-weight starting material, respectively. Table 1 provides guidelines for wet weights of leaf tissue. If using dried starting material the maximum amount which can be processed must be reduced by a factor of approximately 5. Exceeding the recommended maximum amount of starting material will result in inefficient lysis, resulting in low yield and purity.

DNA yields vary depending on genome size, ploidy, and age of sample. Yields typically range from 3–30 µg per 100 mg of wet-weight sample (see "Kit Specifications" on page 5).

Table 1. Approximate wet weights of leaf material

Sample	Size	Approximate wet weight (mg)
Leaf (punch)	1.5 cm diameter	25–75
Leaf (surface area)	12 cm^2 (e.g. 4 x 3 cm)	170–510

Disruption of plant material

Disruption of starting material is an essential step in DNA isolation from plant and fungal material. Insufficient disruption of starting material will lead to low yield and purity. There are a number of disruption methods commonly in use. The most universal method is grinding with a mortar and pestle which can be used for all types of starting material and generally gives optimal results.

A large number of modified protocols are available for disruption of small plant-tissue samples in a microcentrifuge tube. Most of them are adapted for a specific tissue (mostly young leaves). Examples include crushing tissue in liquid nitrogen with a glass rod, plastic

pestle, or wooden stick. Some fresh tissues can be disrupted with a plastic pestle without using liquid nitrogen either in the presence or absence of lysis buffer.

A good alternative is the use of a mixer mill. Samples are disrupted in a mixer mill by mechanical disruption in approximately 1–2 min in the presence of tungsten carbide, steel, or glass beads. When using a mixer mill to disrupt fresh or frozen plant tissue, the plant sample, beads, and tubes should be first cooled in liquid nitrogen. Glass beads can be used when disrupting dried plant or fungal tissue. QIAGEN offers the Mixer Mill MM 300 for processing up to 2 x 24 samples in microcentrifuge tubes in parallel, or in up to two 96-well blocks (see ordering information, page 24). Larger tubes can be used for larger amounts of starting material.

In our hands, optimal yields were obtained with a mortar and pestle or mixer mill. The efficiency of other methods used depends on the starting material e.g., fibrous or non-fibrous material, leaves or needles, young or old tissue etc. Yields with some methods can be only 20–30% of those obtained using a mortar and pestle.

Lysate filtration with QIAshredder

In the DNeasy Plant procedure, cell debris and salt precipitates are removed by centrifugation through a QIAshredder spin column. The preparation of a cleared lysate is essential to prevent clogging of the DNeasy spin column in the following step. Traditional methods involve removing the debris and precipitates by centrifugation and using the supernatant in subsequent steps. However, not all particulate matter forms a compact pellet, making preparation of a cleared lysate by centrifugation very difficult. The QIAshredder spin column removes all cell debris and precipitates making the preparation of a cleared lysate rapid and efficient.

However, with some starting materials (e.g., oak leaves) centrifugation of the entire lysate through the QIAshredder spin column has resulted in sheared DNA. Further investigation has shown that this is not due to the pore size of the QIAshredder spin column, but due to the high viscosity of the lysate and the large amount of precipitates. These form a compact layer on the QIAshredder spin column. Centrifugation of the lysate through this layer can result in size reduction of the DNA. Therefore, for certain plant tissues an additional centrifugation step is recommended. This additional centrifugation is part of the DNeasy Plant Maxi procedure and is included in the DNeasy Plant Mini procedure as an optional step.

Elution

Purified DNA is eluted from the DNeasy spin column using either Buffer AE or water, preheated to 65°C. Optimal results are obtained by eluting twice. The elution volume is typically 2 x 100 µl for the DNeasy Plant Mini Kit and 2 x 750 µl for the DNeasy Plant Maxi Kit.

Higher concentrations of DNA

DNeasy Plant Mini Procedure: If higher concentrations of DNA are required in the eluate, reducing the elution volume to 2 x 50 µl significantly increases concentration but reduces overall yield by approximately 10–30%. If larger amounts (>20 µg) of DNA are loaded onto the column, eluting with 2 x 200 µl will increase yield (see Table 2 on page 14). DNeasy Plant Maxi Procedure: If higher concentrations of DNA are required in the eluate, reducing the elution volume to 2 x 500 µl significantly increases concentration but reduces overall yield. If larger amounts (>100 µg) of DNA are loaded onto the column, eluting with 2 x 1000 µl will increase yield (see Table 3 on page 14).

Preventing dilution of the eluate

DNeasy Plant Mini Procedure: To prevent dilution of the first eluate, the second elution can be performed using a fresh tube. More than 200 µl should not be eluted into a 1.5 ml collection tube because the DNeasy column will contact the eluate, leading to possible aerosol formation of samples during centrifugation.

DNeasy Plant Maxi Procedure: The first eluate contains approximately 70% of the total DNA yield. Therefore, to prevent dilution of the first eluate the second elution can be performed separately. To elute separately, the first eluate can be transferred to a microcentrifuge tube and the 50 ml collection tube can be reused to collect the second eluate. Alternatively, since DNeasy Maxi spin columns are designed to fit into most commercially available 50-ml polypropylene tubes a new tube can be used to collect the second eluate.

Elution with buffer of pH lower than 9.0 may reduce DNA yield. For long-term storage of DNA we recommend eluting in Buffer AE because DNA stored in water is subject to acid hydrolysis.

Table 2. Elution volumes used and corresponding yields with the DNeasy Plant Mini Kit

Source (100 mg young leaves)	Elution volume (µl)	Total DNA yield (µg)*	Concentration (ng/µl)
Arabidopsis	2 x 50	3.6	38
	2 x 100	3.8	20
	2 x 200	4.1	11
Barley	2 x 50	7.9	83
	2 x 100	9.5	50
	2 x 200	10.0	26
Tobacco	2 x 50	20.5	216
	2 x 100	23.2	122
	2 x 200	29.7	78

Elution was performed using either 2 x 50, 2 x 100, or 2 x 200 µl of Buffer AE and the eluates were combined.

** Because the column has a certain residual volume, the volume of eluate recovered is always less than the volume of buffer used for elution; the actual yield is therefore less than the theoretical yield.*

Table 3. Elution volumes used and corresponding yields with the DNeasy Plant Maxi Kit

Source (1 g young leaves)	Elution volume (µl)	Total DNA yield (µg)*	Concentration (ng/µl)
Maize	2 x 500	92	102
	2 x 750	112	79
	2 x 1000	143	72
Fir	2 x 500	63	72
	2 x 750	90	65
	2 x 1000	93	48
Rape	2 x 500	21	23
	2 x 750	26	18
	2 x 1000	27	14

Elution was performed using either 2 x 500, 2 x 750, or 2 x 1000 µl of Buffer AE and the eluates were combined.

** Because the column has a certain residual volume, the volume of eluate recovered is always less than the volume of buffer used for elution; the actual yield is therefore less than the theoretical yield.*

Protocol for Isolation of DNA from Plant Tissue with the DNeasy Plant Mini Kit

Important notes before starting

- If using DNeasy Plant Mini Kits for the first time please read "Technical Information" (page 11).
- Buffers AP1 and AP3/E concentrate may form precipitates upon storage. If necessary, warm to 65°C to redissolve (before adding ethanol to Buffer AP3/E). Do not heat Buffer AP3/E after ethanol has been added
- Buffer AP1 may develop a yellow color upon storage. This does not affect the procedure.
- Buffers AW and AP3/E are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 65°C.
- Preheat Buffer AE to 65°C.
- All centrifugation steps are carried out at room temperature in a microcentrifuge.

1. **Grind plant or fungal tissue under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Continue immediately with step 2.**

   Note: See "Disruption of plant material" (page 11).

2. **Add 400 µl of Buffer AP1 and 4 µl of RNase A stock solution (100 mg/ml) to a maximum of 100 mg of ground (wet weight) or 20 mg (dried) plant or fungal tissue and vortex vigorously.**

   No tissue clumps should be visible. Vortex or pipet further to remove any clumps. Clumped tissue will not lyse properly and will therefore result in a lower yield of DNA. In the rare case where clumps cannot be removed by pipetting and vortexing, a disposable micropestle may be used.

   Note: Do not mix Buffer AP1 and RNase A prior to use.

3. **Incubate the mixture for 10 min at 65°C. Mix 2–3 times during incubation by inverting tube.**

   This step lyses the cells.

Protocol

4. **Add 130 µl of Buffer AP2 to the lysate, mix, and incubate for 5 min on ice.**

   This step precipitates detergent, proteins, and polysaccharides.

   (Optional) Centrifuge the lysate for 5 min at full speed.

   Some plant materials can generate very viscous lysates and large amounts of precipitates during this step resulting in shearing of the DNA in the next step (see "Lysate filtration with QIAshredder", page 12). In this case optimal results are obtained if the majority of these precipitates are removed by centrifugation for 5 min at maximum speed. After centrifugation, apply supernatant to QIAshredder spin column and continue with step 5.

5. **Apply the lysate to the QIAshredder spin column (lilac) sitting in a 2 ml collection tube and centrifuge for 2 min at maximum speed.**

   It may be necessary to cut the end off the pipette tip to apply the lysate to the QIAshredder column. QIAshredder removes most precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet in step 6.

6. **Transfer flow-through fraction from step 5 to a new tube (not supplied) without disturbing the cell-debris pellet.**

   Typically 450 µl of lysate is recovered. For some plant species less lysate is recovered. In this case determine volume for the next step.

7. **Add 1.5 volumes of Buffer AP3/E to the cleared lysate and mix by pipetting.**

   Example: To 450 µl lysate add 675 µl Buffer AP3/E. Reduce the amount of Buffer AP3/E accordingly if less lysate is recovered. A precipitate may form after the addition of ethanol but this will not affect the DNeasy procedure.

   Note: Ensure ethanol has been added to Buffer AP3/E (see "Important notes before starting").

   Note: It is important to pipet Buffer AP3/E directly onto the cleared lysate and to mix immediately.

8. **Apply 650 µl of the mixture from step 7, including any precipitate which may have formed, to the DNeasy mini spin column sitting in a 2 ml collection tube (supplied). Centrifuge for 1 min at ≥6000 x *g* (corresponds to ≥8000 rpm for most microcentrifuges) and discard flow-through.***

   Reuse the collection tube in step 9.

9. **Repeat step 8 with remaining sample. Discard flow-through* and collection tube.**

10. **Place DNeasy column in a new 2 ml collection tube (supplied), add 500 µl Buffer AW to the DNeasy column and centrifuge for 1 min at ≥6000 x *g* (≥8000 rpm). Discard flow-through and reuse the collection tube in step 11.**

    Note: Ensure ethanol is added to Buffer AW (see page 15).

** Flow-through fractions contain Buffer AP3/E, and are therefore not compatible with bleach.*

11. **Add 500 µl Buffer AW to the DNeasy column and centrifuge for 2 min at maximum speed to dry the membrane.**

    It is important to dry the membrane of the DNeasy column since residual ethanol may interfere with subsequent reactions. This spin ensures that no residual ethanol will be carried over during elution. Discard flow-through and collection tube.

    After washing with Buffer AW, the DNeasy mini spin column membrane is usually only slightly colored. In the rare case that the membrane remains significantly colored after washing with Buffer AW, refer to "Darkly colored membrane" in the Troubleshooting Guide on page 21.

    Note: Following the spin, remove the DNeasy column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

12. **Transfer the DNeasy column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied) and pipet 100 µl of preheated (65°C) Buffer AE directly onto the DNeasy membrane. Incubate for 5 min at room temperature and then centrifuge for 1 min at ≥6000 x *g* (≥8000 rpm) to elute.**

    Elution with 50 µl (instead of 100 µl) increases the final DNA concentration in the eluate significantly, but also reduces overall DNA yield. If larger amounts of DNA (>20 µg) are loaded, eluting with 200 µl (instead of 100 µl) increases yield. See "Elution" on page 12.

13. **Repeat elution (step 12) once as described.**

    A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the microcentrifuge tube can be reused for the second elution step to combine the eluates.

    Note: More than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the DNeasy column will contact the eluate.

Protocol for Isolation of DNA from Plant Tissue with the DNeasy Plant Maxi Kit

Important Notes Before Starting

- If using DNeasy Plant Maxi Kits for the first time please read "Technical Information" (page 11).
- Buffers AP1 and AP3/E concentrate may form precipitates upon storage. If necessary, warm to 65°C to redissolve (before adding ethanol to Buffer AP3/E). Do not heat Buffer AP3/E after ethanol has been added.
- Buffer AP1 may develop a yellow color upon storage. This does not affect the procedure.
- Buffers AW and AP3/E are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 65°C.
- Preheat Buffer AE to 65°C.
- All centrifugation steps are carried out at room temperature in a laboratory centrifuge with a **swing-out rotor**. Centrifugation should be at 3000–5000 x *g*, although 5000 x *g* is preferred. Do not use a fixed angle rotor. For further information see "Reagents and Equipment to Be Supplied by User" on page 7.

1. **Grind plant or fungal tissue (maximum 1 g wet weight or 0.2 g dried weight) in liquid nitrogen to a fine powder using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to a 15 ml centrifuge tube (not supplied) and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Continue immediately with step 2.**
2. **Add 5 ml of Buffer AP1 (preheated to 65°C) and 10 µl of RNase A stock solution (100 mg/ml) to a maximum of 1 g of ground tissue and vortex vigorously.**

   No tissue clumps should be visible. Vortex or pipet further to remove any clumps. Clumped tissue will not lyse properly and will therefore result in lower DNA yields.

   Note: Do not premix Buffer AP1 and RNase A prior to use.
3. **Incubate the mixture for 10 min at 65°C. Mix 2–3 times during incubation by inverting the tube.**

   This step lyses the cells.
4. **Add 1.8 ml of Buffer AP2 to the lysate, mix, and incubate for 10 min on ice.**

   This step precipitates detergent, proteins, and polysaccharides.
5. **Spin lysate at 3000–5000 x *g* for 5 min at room temperature.**

   A pellet will form, but some particles will also float.

6. **Decant supernatant into the QIAshredder maxi spin column (lilac) placed in a 50-ml collection tube and spin at 3000–5000 x *g* for 5 min at room temperature in a swing-out rotor. Transfer flow-through, without disturbing the pellet in the collection tube, to a new 50 ml tube (not supplied), and record the volume.**

   Typically, 5–6 ml of lysate is recovered. After centrifugation of the sample most of the debris and precipitates will be retained in the filter but there will also be a pellet in the collection tube. Avoid disturbing the pellet when transferring the supernatant.

7. **Add 1.5 volumes of Buffer AP3/E (see "Important notes before starting") directly to the cleared lysate and mix immediately by vortexing.**

   For example, to 5 ml of cleared lysate add 7.5 ml of Buffer AP3/E. Reduce the amount of Buffer AP3/E accordingly if the volume of lysate is smaller. A precipitate may form after the addition of Buffer AP3/E but this does not affect the DNeasy procedure.

   Note: Ensure ethanol has been added to Buffer AP3/E (see "Important notes before starting").

   Note: It is important to pipet the Buffer AP3/E mixture directly into the cleared lysate and to mix immediately.

8. **Apply sample to the DNeasy maxi spin column (colorless spin column) including any precipitate which may have formed (maximum loading volume 15 ml). Centrifuge at 3000–5000 x *g* for 5 min. Discard flow-through and reuse collection tube.**

9. **Add 12 ml Buffer AW to the DNeasy maxi spin column and centrifuge for 10 min at 3000–5000 x *g* to dry the membrane. Discard flow-through and collection tube.**

   It is important to dry the membrane of the DNeasy maxi spin column since residual ethanol may interfere with subsequent reactions. This spin ensures that no residual ethanol will be carried over during elution.

   After washing with Buffer AW, the DNeasy maxi spin column membrane is usually only slightly colored. In the rare case that the membrane remains significantly colored after washing with Buffer AW, refer to "Darkly colored membrane" in the Troubleshooting Guide on page 21.

10. **Transfer the DNeasy maxi spin column to a new 50 ml tube (supplied). Pipet 0.75–1 ml of Buffer AE (preheated to 65°C) directly onto the DNeasy maxi spin column membrane and leave for 5 min at room temperature. Centrifuge for 5 min at 3000–5000 x *g* to elute.**

    Note: Elution may also be performed with 0.5 ml of Buffer AE (instead of 0.75–1 ml). This increases the final DNA concentration in the eluate, but also reduces overall DNA yield. See "Elution", page 12.

11. **Add another 0.75–1 ml of Buffer AE (preheated to 65°C) and repeat the elution step as described in step 10.**

    The first and second eluates may be combined or collected separately. For separate collection of the eluates, see "Elution" on page 12.

Troubleshooting Guide

		Comments and suggestions
Clogged QIAshredder spin column		
Mini only	a) High viscosity of lysate, precipitates	Perform the optional centrifugation step before loading a large amount of the lysate onto the QIAshredder, as outlined in step 4 of the DNeasy Plant Mini Protocol.
	b) Insufficient centrifugation	Increase the *g*-force and centrifugation time.
Clogged DNeasy spin column		
	a) Carry-over of particulate material	In future preparations, ensure no particulate material is transferred following the centrifugation through QIAshredder.
	b) Lysate too viscous	Reduce the amount of starting material, and/or increase the amounts of Buffers AP1 and AP2.
	c) Insufficient centrifugation	Increase the *g*-force and centrifugation time.
Low yield		
	a) Insufficient disruption	See "Disruption of plant material", page 11. starting material
	b) Insufficient lysis	Reduce the amount of starting material and/or increase starting material the amounts of Buffers AP1 and AP2.
	c) Incorrect binding	Make sure the binding conditions are adjusted correctly in step 7 by accurately determining the amount of lysate recovered.
	d) DNA still bound to the membrane	Increase the volume of Buffer AE or water to 200 µl column (DNeasy Plant Mini Kit) or to ≥1 ml (DNeasy Plant Maxi Kit), and incubate on the column for 5 min at room temperature prior to centrifugation.
Maxi only	e) Incorrect centrifugation method	Fixed angle rotor used instead of swinging-bucket rotor.

		Comments and suggestions
DNA sheared		
	a) Precipitate has formed in Buffer AP1	Ensure that any precipitate that has formed in Buffer AP1 is completely dissolved before use, by heating to 65°C if necessary.
Mini only	b) Debris and precipitates in lysate	Perform the optional centrifugation step before loading a large amount of the lysate onto the QIAshredder, as outlined in step 4 of the DNeasy Plant Mini Protocol.
Darkly colored membrane or green/yellow eluate after washing with Buffer AW		
Mini only	a) Insufficient washing of the membrane	After washing with Buffer AW (step 11), perform an additional wash with 500 µl ethanol (96–100%). Spin for 2 minutes at maximum speed to dry the membrane. Continue with step 12 of the DNeasy Plant Mini Protocol.
Maxi only	b) Insufficient washing of the membrane	After washing with Buffer AW (step 9), perform an additional wash with 12 ml ethanol (90% v/v in water). Spin for 10 min at 3000–5000 *g* and dry the column for 15–30 min at 65°C in an oven to remove residual ethanol. Continue with step 10 in the DNeasy Plant Maxi protocol.
	c) Too much starting material	Reduce the amount of starting material in future preparations.
DNA does not perform well in downstream experiments		
	a) Ethanol carryover	Ensure that during the second wash with Buffer AW, the column is spun at maximum speed for 2 min (mini) or for 10 min at 3000–5000 x *g* (maxi) to dry the membrane. Following the spin, remove the DNeasy column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.
	b) Salt carryover	Ensure Buffer AW is at room temperature before use.

Appendix

Determination of yield and purity

The concentration and purity of DNA can be determined by measuring the absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) in a spectrophotometer. Absorbance readings should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. An absorbance of 1.0 at 260 nm corresponds to 50 µg of DNA per ml (A_{260} = 1 = 50 µg/ml). We recommend to scan absorbance from 220–320 nm as this will indicate whether other factors are interfering with absorbance at 260 and 280 nm.

DNA samples from plant tissue often contain copurified polysaccharides and other metabolites which can interfere with OD readings. Absorbance scans should show a symmetric peak at 260 nm and have an overall smooth shape (Figure 1). Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9.

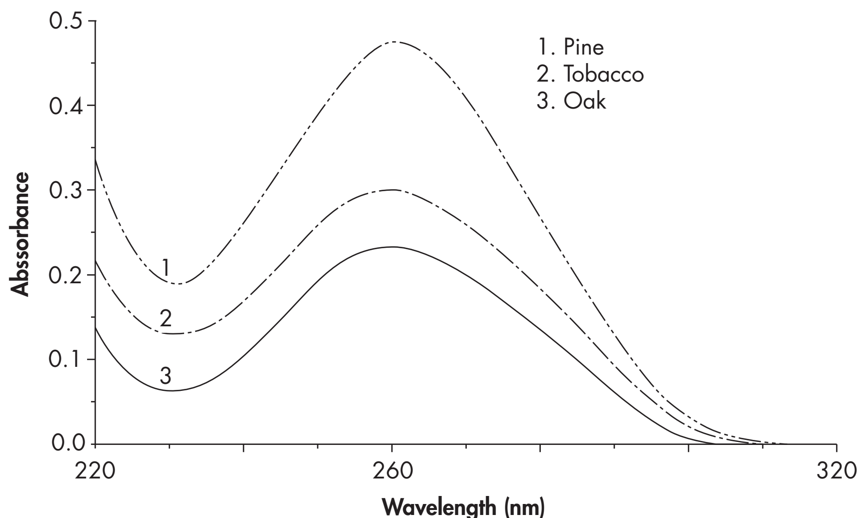


Figure 1. *UV scan of DNeasy purified DNA diluted 1:5 in water.*

If present in the same sample, both DNA and RNA will be measured with a spectrophotometer. If DNA alone is to be quantified in a sample which also contains RNA, a fluorimeter must be used. DNA purified using the DNeasy Plant procedure is free of RNA contamination since an RNase digestion step is included in the procedure.

Average DNA yields obtained with DNeasy Plant Kits

Source		Yield (µg DNA) per 100 mg*
Arabidopsis	*Arabidopsis thaliana*	3–4
Barley	*Hordeum vulgare*	8–12
Fir	*Abies alba*	6–10
Maize	*Zea mays*	15–20
Oak	*Quercus robur*	10–15
Pine	*Pinus sylvestris*	20–25
Potato	*Solanum tuberosum*	4–6
Rape	*Brassica napus*	2–4
Spinach	*Spinacia oleracea*	5–10
Tobacco	*Nicotiana tabacum*	20–25
Tomato	*Lycopersicon esculentum*	10–15
Wheat	*Triticum aestivum*	25–30

** DNA yields vary due to genome size, ploidy, age of sample, etc. All material was collected as young leaves or needles.*

Determination of length

The precise length of genomic DNA should be determined by pulse-field gel electrophoresis (PFGE) through an agarose gel. To prepare the sample for PFGE the DNA should be concentrated by alcohol precipitation, and the DNA pellet dried briefly at room temperature for 5–10 min. Avoid drying the DNA pellet for more than 10 min since overdried genomic DNA is very difficult to redissolve. Redissolve in approximately 30 µl TE buffer, pH 8.0, for at least 30 min at 60°C. Load 3–5 µg of DNA per well. Standard PFGE conditions are as follows: 1% agarose gel in 0.5 x TBE electrophoresis buffer; switch intervals = 5–40 sec; run time = 17 hr; voltage = 170 V.

Ordering Information

Product	Contents	Cat. No.
DNeasy Plant Mini Kits — for isolation of DNA from up to 100 mg of plant tissue		
DNeasy Plant Mini Kit (20)	20 DNeasy Mini Spin Columns, 20 QIAshredder Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69103
DNeasy Plant Mini Kit (50)	50 DNeasy Mini Spin Columns, 50 QIAshredder Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69104
DNeasy Plant Mini Kit (250)	250 DNeasy Mini Spin Columns, 250 QIAshredder Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69106
DNeasy Plant Maxi Kits — for isolation of DNA from up to 1 g of plant tissue		
DNeasy Plant Maxi Kit (6)	6 DNeasy Maxi Spin Columns, 6 QIAshredder Maxi Spin Columns, RNase A, Buffers, Collection Tubes (50 ml)	68161
DNeasy Plant Maxi Kit (24)	24 DNeasy Maxi Spin Columns, 24 QIAshredder Maxi Spin Columns, RNase A, Buffers, Collection Tubes (50 ml)	68163
Accessories		
Buffer AW	324 ml Wash Buffer Concentrate for 1000 preps	19072
Buffer AE	240 ml Elution Buffer for 1000 preps	19077
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
Mixer Mill MM 300*	Universal laboratory mixer mill, 100–115 V/50–60 Hz	85110
Mixer Mill Adapter Set 2 x 24†	2 Sets of Adapter Plates and 2 racks for use with 1.5 or 2.0 ml microcentrifuge tubes on the Mixer Mill MM 300	69998

* *Mixer Mill MM 300 is not available in all countries*
† *Adapter sets are exclusively available from QIAGEN*

Ordering Information

Product	Contents	Cat. No.
Mixer Mill Adapter Set 2 x 96[†]	2 Sets of Adapter Plates for use with collection microtube racks on the Mixer Mill MM 300	69999
Tungsten Carbide Beads, 3 mm (200)[‡]	Tungsten Carbide Beads, suitable for use with 1.2 ml Collection Microtubes	69997
Related Products		
DNeasy 96 Plant Kit for high-throughput isolation of DNA from plant tissue		
DNeasy 96 Plant Kit (6)[§]	For 6 x 96 DNA minipreps: 6 DNeasy 96 Plates, Buffers, Reagents, RNase A, Square-Well Blocks, Collection Microtubes (1.2 ml), Caps, AirPore Tape Sheets	69181
RNeasy Plant Kits — for isolation of up to 100 µg of total RNA from plants and fungi		
RNeasy Plant Mini Kit (20)	20 RNeasy Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5 and 2 ml), RNase-free Reagents and Buffers	74903
RNeasy Plant Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 and 2 ml), RNase-free Reagents and Buffers	74904

[†] *Adapter sets are exclusively available from QIAGEN*

[‡] *Other disruption vessels and beads are available from F. Kurt Retsch GmbH & Co. KG, Haan, Germany or their local distributor*

[§] *Requires use of the QIAGEN 96-Well-Plate Centrifugation System.*

Notes

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