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DNeasy® Plant Pro Kit Handbook

For the isolation of genomic DNA from plant cells, tissues, and seed samples; removes polyphenolics and polysaccharides

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy Plant Pro Kit	**(50)**	**(250)**
Catalog no.	**69204**	**69206**
Number of preps	**50**	**250**
Tissue disruption tubes	50	250
Solution CD1	40 ml	200 ml
MB Spin Columns	50	250
Solution PS	2 x 1.5 ml	14 ml
Solution CD2	15 ml	60 ml
Buffer APP*	35 ml	145 ml
Buffer AW1 (concentrate)*†	19 ml	151 ml
Buffer AW2 (concentrate)†	17 ml	68 ml
Buffer EB	15 ml	55 ml
Collection tubes (1.5 ml)	150	750
Collection tubes (2 ml)	50	250
Quick-Start Protocol	1	1

*Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 4 for safety information.

† Buffer AW1 and Buffer AW2 are supplied as concentrates. Add ethanol (96–100%) according to the bottle label before use to obtain a working solution.

Storage

Solution CD2 should be stored at 2–8°C upon arrival. All other reagents and kit components should be stored at room temperature (15–25°C), until the expiry date printed on the box label.

Intended Use

DNeasy Plant Pro Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

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

Buffer APP and Solution AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a 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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of DNeasy Plant Pro Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy Plant Pro Kit comprises a novel and proprietary method for fast and easy purification of total cellular DNA from plant cells, tissues and seeds. In addition, this kit can also be used to purify bacterial, viral and fungal DNA from plant and root samples. The DNeasy Plant Pro Kit uses bead beating technology, which replaces cumbersome DNA isolation procedures such as CTAB, phenol or chloroform extraction for the recovery of high-quality DNA from the toughest sample types, including strawberry leaf, grapevine leaf, pine needles and various seed types. The DNeasy Plant Pro Kit uses the second generation of QIAGEN's patented Inhibitor Removal Technology® (IRT) to remove PCR inhibitors, including polysaccharides, polyphenolics, and other secondary metabolites from plant extracts during the isolation process. Improved IRT in combination with improved bead beating and lysis chemistry results in high yields of inhibitor-free DNA that is ready for immediate use in downstream applications, including PCR, qPCR, RAPD analysis, RFLP analysis, Southern Blotting, and next-generation sequencing.

Principle and procedure

The DNeasy Plant Pro Kit is designed to isolate high-quality DNA from 5–100 mg of even the most difficult plant samples.

Samples are added to the tissue disruption tube, which contains a specially shaped bead and a buffer for rapid homogenization. Cell lysis and DNA release occur by mechanical and chemical methods. Released genomic DNA is cleared of PCR inhibitors using QIAGEN's second generation IRT and then captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane and is ready for PCR, qPCR, NGS, and other downstream applications.

Bead-beating options

The DNeasy Plant Pro Kit uses bead beating technology that has been optimized for use with a high-velocity bead beater. If desired, a mortar and pestle can still be used in combination with bead beating; this can increase DNA yield from difficult seed samples, for example. The kit contains tissue disruption tubes that are already equipped with a ballcone-shaped bead suitable for high-powered bead beating and may be used with either the TissueLyser II (cat. no. 85300) or the PowerLyzer 24 Homogenizer (cat. no. 13155). In addition, for easier-to-lyse samples, such as soft leaves, a Vortex Adapter may be used; however, this may lead to lower yields.

TissueLyser II system

The TissueLyser II is a highly efficient, convenient bead-beating system that simultaneously disrupts multiple biological samples through high-speed shaking in plastic tubes containing various stainless steel, tungsten carbide, glass, or ceramic beads. Up to 48 or 192 samples can be processed at the same time when using the appropriate adapter set. The TissueLyser II is well suited for mid- to high-throughput disruption of plant materials, including stem, root, seed, or difficult leaf tissue without the need for liquid nitrogen grinding. Lysis using the TissueLyser II system results in reliable and highly reproducible purification of high-quality DNA.

PowerLyzer® 24 Homogenizer (110/220V)

The PowerLyzer 24 Homogenizer is a highly efficient bead-beating system that allows for optimal DNA extraction from plant materials including stem, root, seed, or difficult leaf tissue. The instrument's velocity and proprietary motion provide the fastest homogenization time possible, minimizing the time spent processing samples. The programmable display allows for hands-free, walk-away extraction with up to 10 cycles of bead beating for as long as 5 min per cycle. Even the toughest samples such as pine needles, seeds, spores, and fungal mats are easily and effectively lysed. For more information and protocols, please contact QIAGEN Technical Service at **support.qiagen.com**.

Solution PS

For plant samples high in polyphenolic compounds, we recommend the addition of Solution PS. This solution prevents the loss of nucleic acids by irreversible binding to phenolics, which are removed by the IRT. Examples of plant tissues that require the addition include pine and grape leaves, strawberry leaves, and citrus leaves. Addition of Solution PS to samples that do not require its use is not recommended, because it can lead to reductions in yield. If you are unsure of the phenolic content of your samples, evaluate the DNA recovery with and without using the Solution PS to determine the optimal protocol.

Average DNA yields

DNA yields from plant tissues can vary based on the age and type of tissue and the level of polyphenolic compounds. Table 1 (below) lists estimated DNA yields from a variety of plant samples using the DNeasy Plant Pro Kit.

Table 1. Estimated DNA yields from plant samples using the DNeasy Plant Pro Kit

Plant sample	DNA yield (from 50 mg of sample)	Solution PS
Grape leaf	1–1.5 µg	+
Strawberry leaf	3–5 µg	+
Arabidopsis	1–2 µg	–
Tomato stem	1–1.5 µg	–
Tomato leaf	2.5–3 µg	–
Tomato root	1–2 µg	–
Grass leaf	15–23 µg	–
Pine needle	7–15 µg	+
Walnut	1–2 µg	–
Avocado	0.5–1 µg	–
Mint leaf	2.5–4 µg	–
Apple leaf	1–2 µg	+/–
Apple root	2–4 µg	–
Sunflower seeds	6–14 µg	–
Corn seeds	3–8 µg	–

Note: Positive (+) yields were improved with the addition of Solution PS; negative (–), yields were reduced with the addition of Solution PS; (+/–) yields were equivalent irrespective of Solution PS use.

Automated purification of nucleic acids on QIAcube instruments

Purification of nucleic acids can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the DNeasy Plant Pro Kit for purification of high-quality nucleic acids.

Note: The lyse step is not automated in QIAcube Connect and must be performed manually.

If automating the DNeasy Plant Pro Kit on the QIAcube Connect or the classic QIAcube, the instrument may process fewer than 50 samples due to dead volumes, evaporation, and additional reagent consumption by automated pipetting. QIAGEN only guarantees 50 sample preps when the DNeasy Plant Pro Kit is used manually.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

Figure 1. QIAcube Connect.

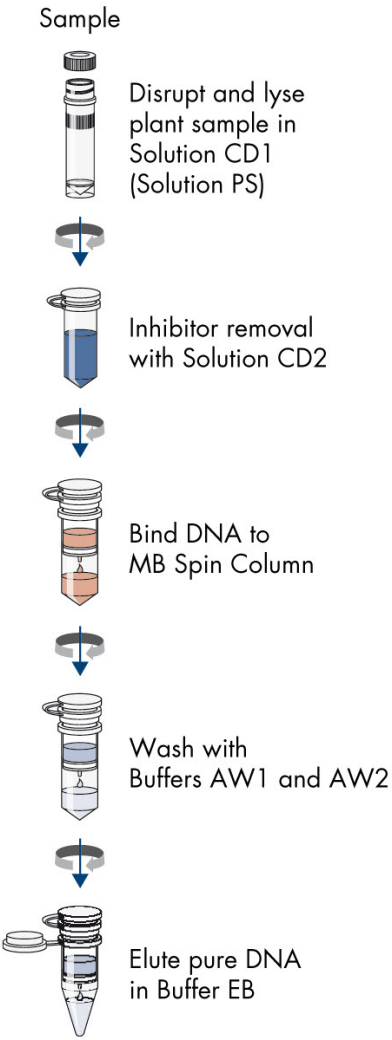

Figure 2. DNeasy Plant Pro Kit Procedure.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Microcentrifuge (capable of up to16,000 x *g*)
- Pipettor (50–600 µl)

Equipment for disruption

- TissueLyser II, cat. no. 85300
- PowerLyzer 24 Homogenizer, cat. no. 13155
- Vortex-Genie® 2 (for vortexing 1.5 or 2 ml tubes, use a Vortex Adapter [cat. no. 13000-V1-24])

Protocol: Purification of Total DNA from Plant Tissue

Important points before starting

- Ensure that the tissue disruption tubes rotate freely in the centrifuge, without rubbing against the sides.
- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Buffer AW1 and Buffer AW2 are supplied as concentrates. Add ethanol (96–100%) according to the bottle label before use to obtain a working solution.
- If Buffer APP has precipitated, heat at 60°C until precipitate dissolves.

Procedure

1. Add 5–100 mg of plant tissue and 500 µl Solution CD1 to a 2 ml tissue disruption tube. Vortex briefly to mix.

   Note: We recommend that the tissue be cut into small pieces before loading into the bead tube. For tough plants or seeds, pregrinding the material with a mortar and pestle may increase yield.

   Note: If your sample is high in phenolic compounds, add 450 µl CD1 and 50 µl Solution PS. For problematic samples, you can add up to 100 µl Solution PS and correspondingly decrease CD1 at this step.

2. Homogenize using one of the following methods:

   Note: Homogenization speed and duration may need to be optimized for your specific sample to ensure highest DNA yield and quality.

A. TissueLyser II:
   Most plant samples can be lysed with the TissueLyser II, using the TissueLyser Adapter Set 2 x 24: Place samples in TissueLyser II and run at 24 Hz for 2 min. Reorient the adapter so that the side that was closest to the machine body becomes furthest from it, and then run the TissueLyser again at 24 Hz for another 2 min.
B. PowerLyzer 24 Homogenizer:
   For the most difficult sample types and highest yields, we recommend the PowerLyzer 24 Homogenizer. Tissue disruption tubes must be properly balanced in the tube holder of the PowerLyzer 24 Homogenizer. Homogenize the sample for 1 cycle at the appropriate speed, depending on sample type, for 1 min.
   Note: We recommend starting at 1700–2000 rpm for most sample types. Excessive speeds may result in tube breakage or leaking (Table 2).

Table 2. Sample types and appropriate PowerLyzer 24 Homogenizer speeds

Plant tissue type	Speed (rpm)
Soft leaf tissue	1500
Fibrous leaf tissue	1700
Stems	1700
Roots	2000
Needles	2100
Seeds	2300

Note: Exceeding these speed limits may result in tube breakage or leaking. Do not increase speed beyond 2400 rpm, otherwise the beads might rupture the tubes.

C. Vortex:
Secure tissue disruption tubes to a Vortex Adapter (cat. no. 13000-V1-24) and vortex at maximum speed for 10 min.

Note: Certain leaf tissues are soft and can be processed for DNA isolation by using a Vortex Adapter. However, plant tissues such as more difficult leaf tissue, roots, wood, needles, and plant seeds require either pregrinding with a mortar and pestle or lysis with a high-powered device.

3. Centrifuge the Tissue Disruption Tubes at 12,000 x *g* for 2 min.
4. Transfer the supernatant to a 1.5 ml collection tube (provided).

   Note: Expect 350–450 µl. The supernatant may still contain some plant particles.
5. Add 200 µl Solution CD2 and vortex for 5 s.

   Note: For problematic samples, you can add up to 250 µl Solution CD2 at this step.
6. Centrifuge at 12,000 x *g* for 1 min at room temperature. Avoiding the pellet, transfer the supernatant to a 1.5 ml collection tube (provided).

   Note: Expect 400–500 µl.
7. Add 500 µl Buffer APP and vortex for 5 s.
8. Load 600 µl of the lysate onto an MB Spin Column and centrifuge at 12,000 x *g* for1 min.
9. Discard the flow-through and repeat step 8 to ensure that all lysate has passed through the MB Spin Column.
10. Carefully place the MB Spin Column into a clean 2 ml collection tube (provided). Avoid splashing any flow-through onto the MB Spin Column.
11. Add 650 µl Buffer AW1 to the MB Spin Column. Centrifuge at 12,000 x *g* for 1 min.
12. Discard the flow-through and place the MB Spin Column back into the same 2 ml collection tube.
13. Add 650 µl Buffer AW2 to the MB Spin Column. Centrifuge at 12,000 x *g* for 1 min.
14. Discard the flow-through and place the MB Spin Column into the same 2 ml collection tube.
15. Centrifuge at up to 16,000 x *g* for 2 min. Carefully place the MB Spin Column into a new 1.5 ml collection tube (provided).
16. Add 50–100 µl of Buffer EB to the center of the white filter membrane.

17. Centrifuge at 12,000 x *g* for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.

    Note: We recommend storing the DNA frozen (–80 to –20°C), because Buffer EB does not contain EDTA. To concentrate DNA, please refer to the "Troubleshooting Guide", page 16.

    Note: Buffer EB is 10 mM Tris (pH 8.0). The DNA bound to the MB Spin Column membrane is resolubilized into Buffer EB.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
DNA	
a) DNA floats out of a well when loading a gel	This usually occurs because residual ethanol remains in the final sample. Avoid transferring any Buffer AW2 to the elution step. Ethanol precipitation (described in "Concentrating eluted DNA", below) is the best way to remove residual ethanol.
b) Concentrating eluted DNA	The final volume of eluted DNA will be 50–100 µl. The DNA may be concentrated by adding 5–10 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 100 µl of 100% cold ethanol and invert 3–5 times to mix. Incubate at –20°C for 30 minutes and centrifuge at 10,000 x *g* for 5 min at room temperature. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid overdrying the pellet or resuspension may be difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris (Buffer EB).
c) Storing DNA	DNA is eluted in Buffer EB (10 mM Tris) and must be stored at –80 to –20°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted in sterile, DNA-free PCR-grade water.
Sample processing	
a) Heating of samples prior to bead beating	Prior to the bead-beating step, the yield of DNA from some plant tissues may be improved by heating at 65°C for 10 min in the presence of Solution CD1 and the optional Solution PS. This varies dramatically between species and portion of the plant. We have found that most leaf, grass, and pine needle samples do not need this heating step. Specific samples will need to be assessed to achieve optimal results.

		Comments and suggestions
b)	Amount of bead beating	Some plant tissues may require more or less bead beating than the recommended settings in "Protocol: Purification of Total DNA from Plant Tissue". This also varies dramatically between species and portion of the plant. Specific samples will need to be assessed to achieve optimal results.
c)	Amount of plant tissue to process	For fresh plant tissues, we recommend starting with 50 mg for most plant types. See Table 1, page 8, for guidelines on the average DNA yields for a variety of plant samples when starting with 50 mg of sample. For lyophilized or dried plant tissues, we recommend using 5–10 mg for most plant types.

Ordering Information

Product	Contents	Cat. no.
DNeasy Plant Pro Kit (50)	Tissue disruption tubes, MB Spin Columns, buffers, collection tubes (1.5 and 2 ml), for 50 preps	69204
DNeasy Plant Pro Kit (250)	Tissue disruption tubes, MB Spin Columns, buffers, collection tubes (1.5 and 2 ml), for 250 preps	69206
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Reagent bottle racks (3), 200 µl filter tips (1024), 1000 µl filter tips (1024), 30 ml reagent bottles (12), rotor adapters (240), rotor adapter holder	990395
Related products		
PowerLyzer 24 Homogenizer	For complete lysis and homogenization of any biological sample	13155
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300
Vortex Adapter	For vortexing 1.5 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
08/2019	Updated storage conditions. Updated text and ordering information for QIAcube Connect.

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