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DNeasy® PowerPlant® Pro Kit Handbook

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

Sample to Insight

QIAGEN

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Kit Contents

DNeasy PowerPlant Pro Kit	**(50)**
Catalog no.	**13400-50**
Number of preps	**50**
PowerBead Tubes, Metal 2.38 mm	50
PowerBead Solution	42 ml
MB Spin Columns	50
Solution SL	2 x 1.5 ml
Solution IR	14 ml
Solution PB	32 ml
Solution CB	30 ml
Solution EB	9 ml
Ethanol	2 x 30 ml
Phenolic Separation Solution	2 x 1.5 ml
RNase A Solution	2 ml
Collection Tubes (2 ml)	3 x 50
Quick Start Protocol	1

Storage

The DNeasy PowerPlant Pro Kit can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All DNeasy products 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.

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.

WARNING 	**Solution CB and ethanol are flammable.**

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

PowerBead Solution and Solution PB 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 PowerPlant Pro Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerPlant Pro Kit is designed for fast and easy purification of total cellular DNA from plant cells, tissues and seeds. The bead beating technology used in this kit 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, cotton leaf, cotton seeds and pine needles. The DNeasy PowerPlant Pro Kit utilizes our Inhibitor Removal Technology® (IRT) to remove PCR inhibitors from plant extracts during the isolation process. The result is inhibitor-free DNA that is ready to use in downstream applications, including PCR, qPCR and sequencing.

Principle and procedure

Plant samples from 5–50 mg are added to a bead tube along with a kit-supplied buffer for rapid homogenization. Cell lysis and DNA release occurs by mechanical and chemical methods. Released genomic DNA is cleared of PCR inhibitors using IRT and then captured on a silica membrane in a spin column format. DNA is washed and eluted from the membrane and ready for PCR and other downstream applications.

Optimized for homogenization with the PowerLyzer 24 Homogenizer

The DNeasy PowerPlant Pro Kit may be used with a vortex or high velocity bead beater, such as the PowerLyzer® 24 Homogenizer. The PowerLyzer 24 Homogenizer is suitable for fast homogenization of plant materials including stems, roots, seeds or difficult leaf tissue without the need of liquid nitrogen grinding.

The PowerLyzer 24 Homogenizer is a highly efficient bead beating system that allows for optimal DNA extraction from a variety of plant tissues. The instrument's velocity and proprietary motion combine to 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 ten cycles of bead beating for as long as 5 minutes per cycle.

This kit provides PowerBead Tubes prefilled with 2.38 mm stainless steel beads for homogenizing plant tissue for optimal DNA isolation. Alternative pre-filled bead tube options are available for additional applications. Please contact QIAGEN Technical Service at **support.qiagen.com** for details.

For isolation of DNA from plant tissues using the DNeasy PowerPlant Pro Kit and the PowerLyzer 24, refer to guidelines for getting started in Step 4 on pages 12 and 14 of this handbook.

Using the DNeasy PowerPlant Pro Kit with other homogenizers

To isolate DNA using the DNeasy PowerPlant Pro Kit with FastPrep® or Precellys® homogenizers, use the conversion chart shown in Table 1 (Page 8) to adapt your current protocol. However, due to the highly efficient motion of beads in the PowerLyzer 24 Homogenizer, fewer cycles are required to generate the same effect using it compared to other homogenizers. You may want to perform extractions using the PowerLyzer 24 Homogenizer at the equivalent speed and number of cycles as your current instrument and then compare the results to those obtained using less time or lower speeds to determine which settings give the best results.

Phenolic Separation Solution

For plant samples high in polyphenolic compounds, we recommend the addition of the Phenolic Separation Solution. This Solution prevents loss of nucleic acids by preventing irreversible binding to phenolics, which are removed by the IRT. Not all plant samples require the addition of the Phenolic Separation Solution. Examples of plant tissues that require the addition includes pine and grape leaf. If you are unsure of the phenolic content of your samples, evaluate the DNA recovery with and without using the Phenolic Separation Solution to determine the optimal protocol.

Table 1. Conversion chart to use the DNeasy PowerPlant Pro Kit with FastPrep or Precellys homogenizers

PowerLyzer 24 Homogenizer	FastPrep 24 (m/s)	Precellys 24
2500	4	5000
2600	–	5200
2700	–	5400
2800	4.5	5600
2900	–	5800
3000	–	6000
3100	5	6200
3200	–	6400
3300	–	6600
3400	5.5	6800
3500	–	–
3600	–	–
3700	6	–
3800	–	–
3900	–	–
4000	6.5	–

Note: Settings equivalent to slower than 2500 RPM or faster than 4000 RPM on the PowerLyzer 24 Homogenizer are not obtainable with FastPrep or Precellys homogenizers.

Average DNA yields

DNA yields from plant tissues can vary based on the age and type of tissue and the level of phenolic compounds. Table 2 (Page 9) lists estimated DNA yields from a variety of plant samples evaluated using the DNeasy PowerPlant Pro Kit.

Table 2. Estimated DNA yields from plant samples using the DNeasy PowerPlant Pro Kit

Plant sample	DNA yield (from 50 mg of sample)	Phenolic Separation Solution (PSS)
Grape leaf	2.5–3.5 µg	+
Strawberry leaf	10–15 µg	+
Tomato stem	10–25 µg	+
Cotton leaf	2.5–3.5 µg	+/–
Cotton stem	20–25 µg	–
Grass leaf	40–50 µg	–
Pine needle	30–35 µg	+
Rice leaf	7–11 µg	–
Mint leaf	2–3 µg	–

Note: Positive (+) yields were improved with the addition of PSS; negative (–), yields were reduced with the addition of PSS; **+/–,** yields were the same with or without PSS.

High-throughput Options

For additional high-throughput options, we offer the DNeasy PowerPlant Pro HTP 96 Kit (cat. no. 13496-4) for processing up to 2 x 96 samples using a centrifuge capable of spinning two stacked 96-well blocks (13 cm x 8 cm x 5.5 cm) at 2500 x *g*. For 96-well homogenization of plant tissue, we offer the TissueLyser II and Plate Adapter Set (cat. no. 85300 and 11990, respectively.)

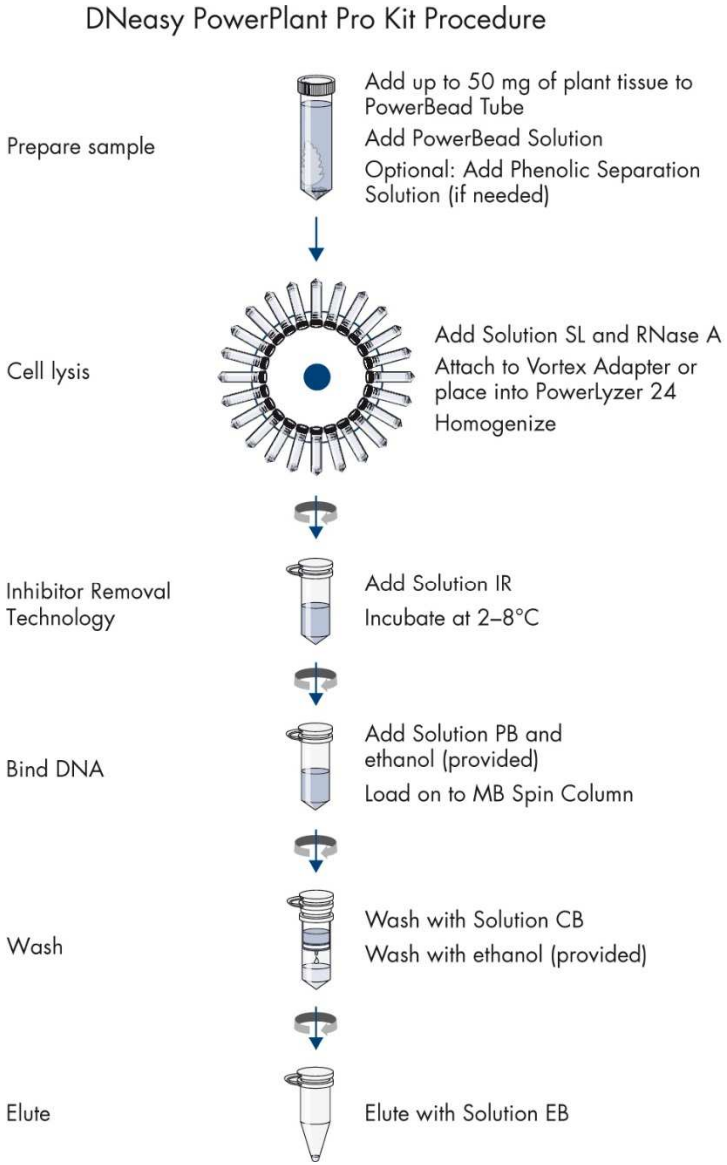

Figure 1. DNeasy PowerPlant Pro Kit procedure.

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 (up to16,000 x *g*)
- PowerLyzer 24 or another bead homogenizer
- Pipettor (50–600 µl)
- Vortex-Genie® 2
- Vortex Adapter for vortexing 1.7 or 2 ml tubes (cat. no. 13000-V1-24)

Protocol: Experienced User

Notes before starting

- If Solution SL contains precipitates, heat at 37–55°C to dissolve precipitates.

Procedure

1. Add up to 50 mg of fresh plant tissue and 450 µl of Bead Solution to a 2 ml PowerBead Tube, Metal 2.38 mm (provided).
   Note: We recommended the tissue be cut into small pieces before loading into the bead tube. For tough plants or seeds, pre-grind the material with a mortar and pestle.
   Note: If sample is high in phenolics and you are using the Phenolic Separation Solution, reduce Bead Solution to 410 µl and add 40 µl of the Phenolic Separation Solution.
2. Add 50 µl of Solution SL and 3 µl of RNase A Solution and vortex briefly to mix.
3. Homogenize using one of the following methods:
   A. **Vortex:**
      Secure PowerBead Tubes horizontally to a Vortex Adapter (cat. no. 13000-V1-24) or on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 min.
      Note: Most leaf tissues are soft and can be processed for DNA isolation by using a Vortex Adapter. However, plant tissues such as roots, wood, and plant seeds require pre-grinding with a mortar and pestle before placing on the vortex.
   B. PowerLyzer 24 Homogenizer:
      1. Properly identify each PowerBead Tube on both the cap and on the side.
         Note: Due to the high energies of the PowerLyzer 24 Homogenizer, marring of the cap tops is possible. Therefore, we recommend you mark the sides of the PowerBead Tubes as well as the caps to ensure proper sample identification.
      2. Place the PowerBead Tubes into the tube holder of the PowerLyzer 24 Homogenizer. The PowerBead Tubes must be balanced properly. Homogenize the tissue for **1 cycle** at the appropriate speed (see Table 3 below) depending on sample type for **2 min**.

Table 3. Sample types and appropriate PowerLyzer 24 Homogenizer speeds

Plant tissue type	Speed (RPM)
Soft leaf tissue	2000
Fibrous leaf tissue	2200
Stems	2200
Roots	2500
Pine needles	2600
Seeds	2800

Note: Exceeding these speed limits may result in tube breakage or leaking.

4. Centrifuge PowerBead Tubes at 13,000 x *g* for 2 min. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
5. Add 175 µl of Solution IR. Vortex for 5 s and then incubate at 2–8°C for 5 min.
   Note: For problematic samples you can add up to 250 µl of Solution IR at this step.
6. Centrifuge at 13,000 x *g* for 2 min. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube (provided).
7. Add 600 µl of Solution PB and 600 µl of ethanol (provided). Vortex for 5 s.
8. Load approximately 600 µl of lysate onto an MB Spin Column and centrifuge at 10,000 x *g* for 30 s. Discard the flow-through and repeat until all the lysate has been passed through the MB Spin Column. Discard the flow-through and place the MB Spin Column back into the Collection Tube.
9. Add 500 µl of Solution CB to the MB Spin Column. Centrifuge at 10,000 x *g* for 30 s. Discard the flow-through and place the Spin Filter back into the same Collection Tube.
10. Add 500 µl of ethanol (provided) to the MB Spin Column. Centrifuge at 10,000 x *g* for 30 s. Discard flow-through and place the Spin Filter back into the same Collection Tube.
11. Centrifuge at up to 16,000 *x g* for 2 min. Carefully place the MB Spin Column into a new 2 ml Collection Tube (provided). Avoid splashing ethanol the MB Spin Column.
12. Add 50–100 µl of Solution EB to the center of the white filter membrane and incubate for 2 min at room temperature.
13. Centrifuge at 10,000 *x g* for 30 s. For maximum elution efficiency, reload the flow-through on to the center of the white filter membrane. Centrifuge 30 s at 10,000 *x g*.
14. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Protocol: Detailed

Notes before starting

- If Solution SL contains precipitates, heat at 37–55°C to dissolve precipitates.

Procedure

1. Add up to 50 mg of fresh plant tissue and 450 µl of Bead Solution to a 2 ml PowerBead Tube, Metal 2.38 mm (provided).
   Note: We recommended the tissue be cut into small pieces before loading into the bead tube. For tough plants or seeds, pre-grind the material with a mortar and pestle.
   Note: If sample is high in phenolics and you are using the Phenolic Separation Solution, reduce Bead Solution to 410 µl and add 40 µl of the Phenolic Separation Solution.
   Note: Plant material is added to the PowerBead Tube to prepare it for a bead-beating homogenization step. The Phenolic Separation Solution disassociates the phenolics from the nucleic acids so that they can be removed during the IRT process.
2. Add 50 µl of Solution SL and 3 µl of RNase A Solution and vortex briefly to mix.
   Note: RNase A will digest unwanted RNA during the homogenization step.
3. Homogenize using one of the following methods:
   A. **Vortex:**
      Secure PowerBead Tubes horizontally to a Vortex Adapter (cat. no. 13000-V1-24) or on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 min.
      Note: Most leaf tissues are soft and can be processed for DNA isolation by using a Vortex Adapter. However, plant tissues such as roots, wood, and plant seeds require pre-grinding with a mortar and pestle before placing on the vortex.
   B. **PowerLyzer 24 Homogenizer:**
      Properly identify each PowerBead Tube on both the cap and on the side.
      Note: Due to the high energies of the PowerLyzer 24, marring of the cap tops is possible. Therefore, we recommend you mark the sides of the PowerBead Tubes as well as the caps to ensure proper sample identification.

Place the PowerBead Tubes into the tube holder of the PowerLyzer 24 Homogenizer. The PowerBead Tubes must be balanced properly. Homogenize the tissue for **1 cycle** at the appropriate speed (see Table 3 below) depending on sample type for **2 min**.

Note: The bead beating step homogenizes plant material without the need for manual grinding. In some cases, the plant material will not be completely disintegrated after the specified times for each method. However, there should be sufficient disruption for good yields of DNA.

Table 3. Sample types and appropriate PowerLyzer 24 Homogenizer speeds

Plant tissue type	Speed (RPM)
Soft leaf tissue	2000
Fibrous leaf tissue	2200
Stems	2200
Roots	2500
Pine needles	2600
Seeds	2800

Note: Exceeding these speed limits may result in tube breakage or leaking.

4. Centrifuge PowerBead Tubes at 13,000 x *g* for 2 min. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: This step pellets unwanted cell and tissue debris. The supernatant contains DNA and other cell components. Avoid transferring any solid plant tissue.
5. Add 175 µl of Solution IR. Vortex for 5 s and then incubate at 2–8°C for 5 min.
   Note: For problematic samples you can add up to 250 µl of Solution IR at this step.
   Note: Solution IR is a novel formulation of Inhibitor Removal Technology and completes the removal of PCR inhibitors in one step.
6. Centrifuge at 13,000 x *g* for 2 min. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube (provided).
   Note: This step pellets unwanted proteins and inhibitors.
7. Add 600 µl of Solution PB and 600 µl of ethanol (provided). Vortex for 5 s.

Note: Solution PB contains a binding salt. The concentration and amount of this salt allows for optimal DNA binding to the silica filter membrane of the MB Spin Column. Ethanol allows for maximal nucleic acid binding to the column.

8. Load approximately 600 µl of lysate onto an MB Spin Column and centrifuge at 10,000 x *g* for 30 s. Discard the flow-through and repeat until all the lysate has been passed through the MB Spin Column. Discard the flow-through and place the MB Spin Column back into the Collection Tube.
   Note: In the presence of Solution PB and ethanol, DNA will bind to the MB Spin Column. Centrifugation of the combined lysate through the MB Spin Column allows DNA to bind to the filter membrane while allowing unwanted salt and impurities to pass through the membrane.
9. Add 500 µl of Solution CB to the MB Spin Column. Centrifuge at 10,000 x *g* for 30 s. Discard the flow-through and place the Spin Filter back into the same Collection Tube.
   Note: Solution CB is a wash buffer that contains ethanol and removes residual salt and other impurities from the MB Spin Column membrane.
10. Add 500 µl of ethanol (provided) to the MB Spin Column. Centrifuge at 10,000 x *g* for 30 s. Discard flow-through and place the spin filter back into the same Collection Tube.
    Note: Ethanol efficiently removes all metabolites and salt from the MB Spin Column membrane.
11. Centrifuge at up to 16,000 *x g* for 2 min. Carefully place the MB Spin Column into a new 2 ml Collection Tube (provided). Avoid splashing ethanol on the MB Spin Column.
12. Add 50–100 µl of Solution EB to the center of the white filter membrane and incubate for 2 min at room temperature.
13. Centrifuge at 10,000 *x g* for 30 s. For maximum elution efficiency, reload the flow-through on to the center of the white filter membrane. Centrifuge 30 s at 10,000 *x g*.
    Note: Solution EB is 10 mM Tris (pH 8.0). The DNA bound to the MB Spin Column membrane is re-solubilized into Solution EB.
14. Discard the MB Spin Column. The DNA is now ready for downstream applications.

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 Solution CB to the elution step. Ethanol precipitation (described in "Concentrating eluted DNA") 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 minutes at room temperature. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris (Solution EB).
c)	Storing DNA	DNA is eluted in Solution EB (10 mM Tris) and must be stored at –20°C to –80°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 (cat. no. 17000-10).
Sample processing		
a)	Heating of samples prior to bead beating	Prior to the bead beating step, some plant tissues may require heating at 65°C for 10 minutes in the presence of PowerBead Solution/Solution SL and the optional Phenolic Separation Solution. 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 starting points stated on pages 12 and 14. 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 on page 7 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 between 5–10 mg for most plant types.

Ordering Information

Product	Contents	Cat. no.
DNeasy PowerPlant Pro Kit (50)	For 50 preps: Isolation of genomic DNA from plant and seed samples, removes polyphenolics and polysaccharides	13400-50
DNeasy PowerPlant Pro HTP 96 Kit (384)	384 preps: High-throughput isolation of genomic DNA from plant and seed samples	13496-4
Related Products		
RNeasy® PowerPlant Kit (50)	For 50 preps: Isolation of total RNA from plant and seed samples, including those high in polyphenols and polysaccharides	13500-50
DNase Max® Kit (384)	For 384 preps: Removal of genomic DNA contamination in RNA preparations using a high activity DNase I enzyme and a highly specific DNase removal resin	15200-50
Vortex Adapter	For vortexing 1.7 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
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

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.

Notes

Notes

Limited License Agreement for DNeasy PowerPlant Pro Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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