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DNeasy® PowerSoil® Pro Kit Handbook

For the isolation of microbial genomic DNA from all soil types, including difficult samples such as compost, sediment and manure

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy PowerSoil Pro Kit	**(50)**	**(250)**
Catalog no.	**47014**	**47016**
Number of preps	**50**	**250**
PowerBead Pro Tubes	50	250
MB Spin Columns	50	250
Solution CD1	40 ml	200 ml
Solution CD2	15 ml	60 ml
Solution CD3	35 ml	175 ml
Solution EA	36 ml	175 ml
Solution C5	32.5 ml	175 ml
Solution C6	9 ml	66 ml
Microcentrifuge Tubes (2 ml)	100	500
Elution Tubes (1.5 ml)	50	250
Collection Tubes (2ml)	100	500
Quick Start Protocol	1	1

Storage

All components and reagents of the DNeasy PowerSoil 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 EA and Solution C5 are flammable.**

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

Solution CD1 and Solution CD3 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 PowerSoil Pro Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerSoil Pro Kit comprises a novel and proprietary method for isolating microbial genomic DNA from environmental samples. The kit uses QIAGEN's second-generation Inhibitor Removal Technology® (IRT), and is intended for use with environmental samples containing high humic acid content, including difficult soil types such as compost, sediment and manure. Other more common soil and stool types have also been used successfully with this kit. Improved IRT combined with more efficient bead beating and lysis chemistry yields high-quality DNA that can be used immediately in downstream applications, including PCR, qPCR and next-generation sequencing (16s and whole-genome).

Principle and procedure

The DNeasy PowerSoil Pro Kit is effective at removing PCR inhibitors from even the most difficult soil types. Environmental samples are added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane and ready for NGS, PCR and other downstream applications.

Bead beating options

The DNeasy PowerSoil Pro Kit does not require homogenization using a high-velocity bead beater. However, if the microorganism of interest requires stronger homogenization than provided by a vortex, or if using a bead beater is desired, the DNeasy PowerSoil Pro Kit contains bead tubes suitable for high-powered bead beating and may be used in conjunction with the PowerLyzer® 24 Homogenizer (110/220V) (cat. no. 13155) or the TissueLyzer II (cat. no. 85300) using a 2 ml Tube Holder Set (cat no. 11993).

The PowerLyzer 24 Homogenizer (110/220V): Optimized for complete homogenization of any sample

The PowerLyzer 24 Homogenizer is a highly efficient bead beating system that allows for optimal DNA extraction from a variety of biological samples. 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. Even the toughest and most difficult 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**.

High-throughput Options

For high-throughput options, we offer the DNeasy PowerSoil HTP 96 Kit (cat. no. 12955-4) 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 soil, we offer the TissueLyser II and Plate Adapter Set (cat. no. 85300 and 11990, respectively.)

Automated nucleic acid purification on the QIAcube

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

If automating the DNeasy PowerSoil Pro Kit on the 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 with manual use of the DNeasy PowerSoil Pro Kit.

For more information about the automated procedure, see the relevant protocol sheet available at **www.qiagen.com/MyQIAcube**. Up-to-date protocol sheets can be downloaded free of charge, or may be obtained by contacting QIAGEN Technical Services at **support.qiagen.com**.

Figure 1. The QIAcube instrument.

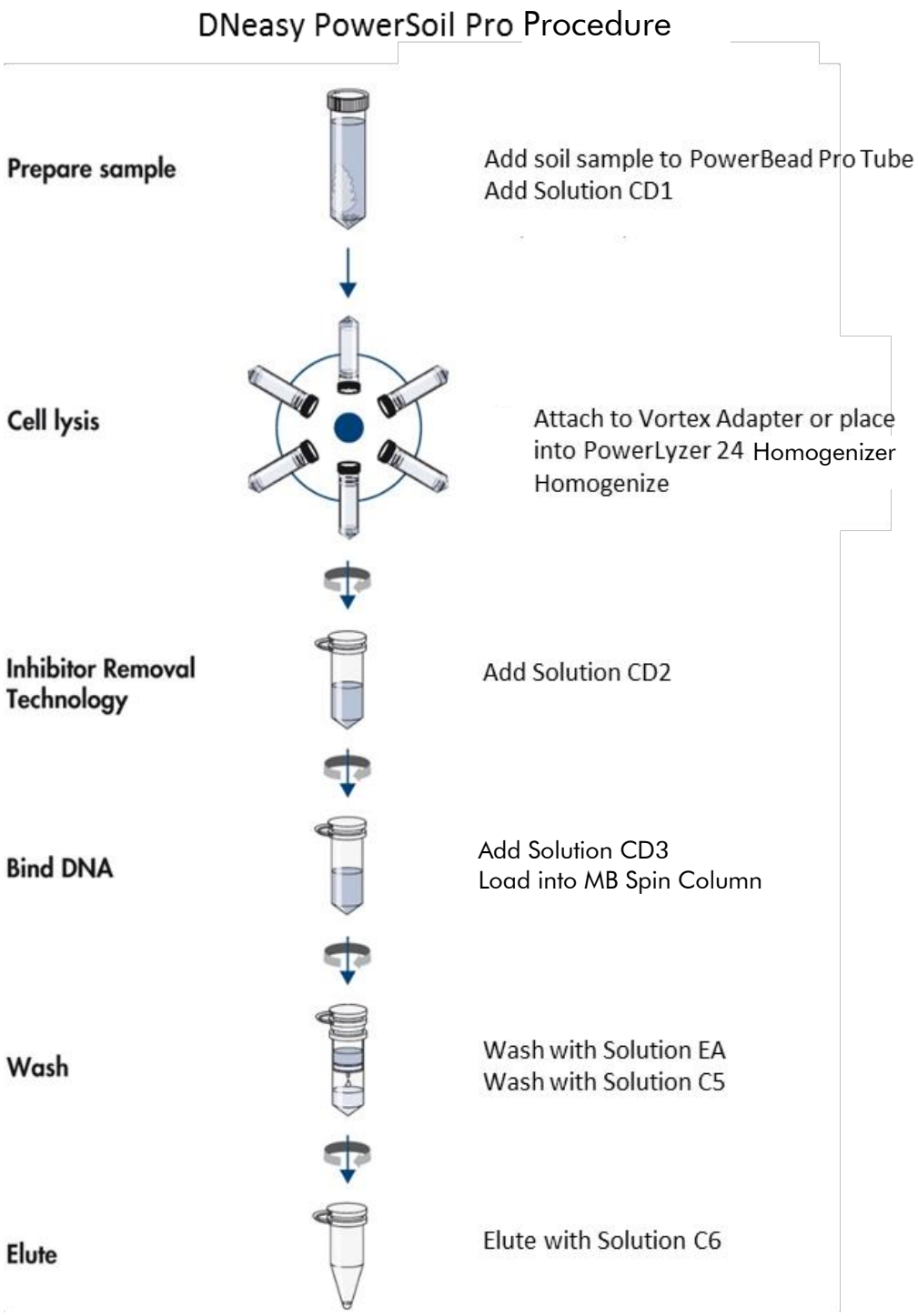

Figure 2. DNeasy PowerSoil Pro Kit procedure.

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*)
- Pipettor (50–1000 µl)
- Vortex-Genie® 2
- Vortex Adapter for 24 (1.5 or 2 ml) tubes (cat. no. 13000-V1-24)

Protocol: Experienced User

Important notes before starting

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).

Procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up to 250 mg of soil and 800 µl of Solution CD1. Vortex briefly to mix.
2. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5– 2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   Note: If using the Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 5–10 min.
3. Centrifuge the PowerBead Pro Tube at 15,000 x *g* for 1 min.
4. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).
   Note: Expect 500–600 µl. The supernatant may still contain some soil particles.
5. Add 200 µl of Solution CD2 and vortex for 5 s.
6. Centrifuge at 15,000 x *g* for 1 min at room temperature. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided).
   Note: Expect 500–600 µl.
7. Add 600 µl of Solution CD3 and vortex for 5 s.
8. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x *g* for 1 min.
9. Discard the flow-through and repeat step 8 to ensure that all of the 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 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 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 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min.
14. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).
15. Centrifuge at up to 16,000 x *g* for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).
16. Add 50–100 µl of Solution C6 to the center of the white filter membrane.
17. Centrifuge at 15,000 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 (–15 to –30°C or –65 to –90°C) as Solution C6 does not contain EDTA. To concentrate DNA, please refer to the Troubleshooting Guide.

Protocol: Detailed

Important notes before starting

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).

Procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up to 250 mg of soil and 800 µl of Solution CD1. Vortex briefly to mix.
   Note: After the sample has been loaded into the PowerBead Pro Tube, the next step is a homogenization and lysis procedure. The PowerBead Pro Tube contains a buffer that will (a) help disperse the soil particles, (b) begin to dissolve humic acids and (c) protect nucleic acids from degradation. Gentle vortexing mixes the components in the PowerBead Pro Tube and begins to disperse the sample in the buffer.
2. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   Note: If using the Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 5–10 min.
   Note: Vortexing is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from step 1 and mechanical shaking introduced at this step. Randomly shaking the beads in the presence of disruption agents will cause the beads to collide with microbial cells and lead to the cells breaking open. Using the Vortex Adapter will maximize homogenization, which can lead to higher DNA yields. Avoid using tape, which can become loose and result in reduced homogenization efficiency, inconsistent results and reduced yields.
3. Centrifuge the PowerBead Pro Tube at 15,000 x *g* for 1 min.
4. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).
   Note: Expect 500–600 µl. The supernatant may still contain some soil particles.

5. Add 200 µl of Solution CD2 and vortex for 5 s.
   Note: Solution CD2 is Inhibitor Removal Technology (IRT). It contains a reagent that can precipitate non-DNA organic and inorganic material including humic substances, cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.
6. Centrifuge at 15,000 x *g* for 1 min at room temperature. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided).
   Note: Expect 500–600 µl.
   Note: The pellet at this point contains non-DNA organic and inorganic material including humic acid, cell debris and proteins. For best DNA yields and quality, avoid transferring any of the pellet.
7. Add 600 µl of Solution CD3 and vortex for 5 s.
   Note: Solution CD3 is a high-concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, Solution CD3 will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the MB Spin Column filter membrane.
8. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x *g* for 1 min.
   Note: DNA is selectively bound to the silica membrane in the MB Spin Column in the presence of high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.
9. Discard the flow-through and repeat step 8 to ensure that all of the 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 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min.
    Note: Solution EA is a wash buffer that removes protein and other non-aqueous contaminants from the MB Spin Column filter membrane.

12. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.
13. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min.
    Note: Solution C5 is an ethanol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the MB Spin Column. This wash solution removes residual salt, humic acid and other contaminants while allowing the DNA to stay bound to the silica membrane.
14. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).
15. Centrifuge at up to 16,000 x *g* for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).
    Note: This spin removes residual Solution C5. It is critical to remove all traces of Solution C5 because the ethanol in it can interfere with downstream DNA applications, such as PCR, restriction digests and gel electrophoresis.
16. Add 50–100 µl of Solution C6 to the center of the white filter membrane.
    Note: Placing Solution C6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wet. This will result in a more efficient and complete release of the DNA from the MB Spin Column filter membrane. As Solution C6 passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris), which lacks salt.
17. Centrifuge at 15,000 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 (–15 to –30°C or –65 to –90°C) as Solution C6 does not contain EDTA. To concentrate DNA, please refer to the Troubleshooting Guide.

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
Soil Processing		
a)	Amount of soil to process	The QIAGEN DNeasy PowerSoil Pro Kit is designed to process 0.25 grams of soil. For inquiries regarding the use of larger sample amounts, please contact Technical Support for suggestions.
b)	Soil Sample is high in water content	Remove contents from the PowerBead Pro Tube (beads) and transfer into another sterile microcentrifuge tube (not provided). Add soil sample to PowerBead Pro Tube and centrifuge at room temperature for 30 seconds at 10,000 x *g*. Remove as much liquid as possible with a pipette tip. Add beads back to PowerBead Pro Tube and resume protocol from step 2.
DNA		
a)	DNA does not amplify	Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will inhibit a PCR reaction. Diluting the template DNA should not be necessary with DNA isolated using the QIAGEN PowerSoil Pro DNA Kit. However, it should still be attempted. If DNA will still not amplify after trying the steps above, then PCR optimization may be needed.
b)	Eluted DNA is brown	If you observe coloration in your samples, please contact Technical Support for suggestions.
c)	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 –15 to –30°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 C6).

		Comments and suggestions
d)	DNA floats out of a well when loading a gel	This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 15 and not transferring liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in "Concentrating eluted DNA") is the best way to remove residual Solution C5.
e)	Storing DNA	DNA is eluted in Solution EB (10 mM Tris) and must be stored at –15 to –30°C or –65 to –90°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).
Alternative lysis methods		
a)	Cells are difficult to lyse	After adding Solution CD1 and prior to the bead beating step, incubate at 65°C for 10 minutes. Resume protocol from step 2.
b)	Reduction of shearing of DNA	After adding Solution CD1, vortex 3—4 seconds, then heat to 70°C for 5 minutes. Repeat once. This alternative procedure will reduce shearing but may also reduce yield.

Ordering Information

Product	Contents	Cat. no.
DNeasy PowerSoil Pro Kit (50)	For 50 preps: Isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For 250 preps: Isolation of microbial genomic DNA from all soil types	47016
Related products		
DNeasy PowerSoil Kit (50)	For 50 preps: Isolate microbial genomic DNA from all soil types	12888-50
DNeasy PowerSoil Kit (100)	For 100 preps: Isolate microbial genomic DNA from all soil types	12888-100
DNeasy PowerMax® Soil Kit (10)	For 10 preps: Isolate microbial DNA from large quantities of soil; great for samples with low microbial load	12988-10
RNeasy® PowerSoil Total RNA Kit (25)	For 25 preps: Isolate high-quality total RNA from all soil types	12866-25
MagAttract® PowerSoil DNA KF Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27000-4-KF
Vortex Adapter	For vortexing 1.5 ml or 2 ml tubes using the Vortex-Genie 2	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.

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Limited License Agreement for DNeasy PowerSoil Pro Kit

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

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