May 2017

DNeasy® PowerSoil® Kit Handbook

For the isolation of microbial genomic DNA from all soil types

Sample to Insight

QIAGEN

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Kit Contents

DNeasy PowerSoil Kit Catalog no. Number of preps	(50) 12888-50 50	(100) 12888-100 100
MB Spin Columns	50	2 x 50
PowerBead Tubes, Garnet	50	2 x 50
Solution C1	6.6 ml	6.6 ml
Solution C2	15 ml	2 x 15 ml
Solution C3	15 ml	2 x 15 ml
Solution C4	72 ml	2 x 72 ml
Solution C5	30 ml	2 x 30 ml
Solution C6	9 ml	2 x 9 ml
Collection Tubes (2 ml)	4 x 50	8 x 50
Quick Start Protocol	1	1

Storage

The DNeasy PowerSoil Kit reagents and components 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.

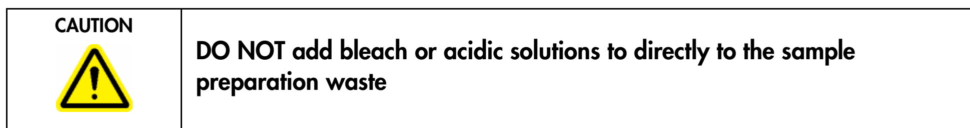
All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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 C5 contains ethanol. It is flammable.

WARNING: Do not use bleach to clean the inside of the QIAvac® 24 Plus manifold.



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

PowerBead Tubes and Solution C4 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 Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerSoil Kit comprises a novel and proprietary method for isolating genomic DNA from environmental samples using patented Inhibitor Removal Technology® (IRT). This kit 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 types have also been used successfully with this kit. The isolated DNA has a high level of purity, which allows for more successful PCR amplification of organisms from the sample. PCR analysis has been performed to detect a variety of organisms including bacteria (e.g., *Bacillus subtilis*, *Bacillus anthracis*), fungi (e.g., yeasts, molds), algae and actinomycetes (e.g., *Streptomyces*).

Principle and procedure

The DNeasy PowerSoil Kit uses a humic substance/brown color removal procedure. This procedure 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. The isolated DNA is ready for PCR analysis and other downstream applications.

Bead beating options

The DNeasy PowerSoil 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 Kit may be used in conjunction with the PowerLyzer® 24 Homogenizer (110/220V) (cat. no. 13155). We now offer DNeasy PowerLyzer PowerSoil Kits (cat. no. 12855-50 and 12855-100) with bead tubes suitable for high-powered bead beating of soil. For more information about these

products, or for references using the DNeasy PowerSoil Kit with other bead mill instruments, please contact Technical Support at **support.qiagen.com**.

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

The PowerLyzer 24 Homogenizer (110/220V) is a bead beating instrument uniquely designed for the most efficient and complete lysis and homogenization of biological samples. In as little as 30 seconds, the PowerLyzer 24 Homogenizer is capable of processing up to 24 samples in 2 ml tubes. With true 'hands-free' operation, the downtime associated with manipulating samples through multiple cycles is eliminated. Even the toughest and most difficult samples such as pine needles, seeds, spores, fungal mats and clay soils are easily and effectively lysed. For more information and protocols, please contact Technical Support at **support.qiagen.com**.

High-throughput options

We offer a vacuum-based protocol for faster processing without centrifugation for the DNA-binding and column-washing steps for MB Spin Columns. The QIAvac 24 Plus Manifold allows for processing of up to 24 MB Spin Column preps at a time. For additional high-throughput options, we offer the DNeasy PowerSoil HTP 96 Kit for processing up to 2 x 96 samples using a centrifuge capable of spinning two 96 well blocks stacked (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 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 Kit for purification of high-quality DNA.

If automating the DNeasy PowerSoil 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 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**.

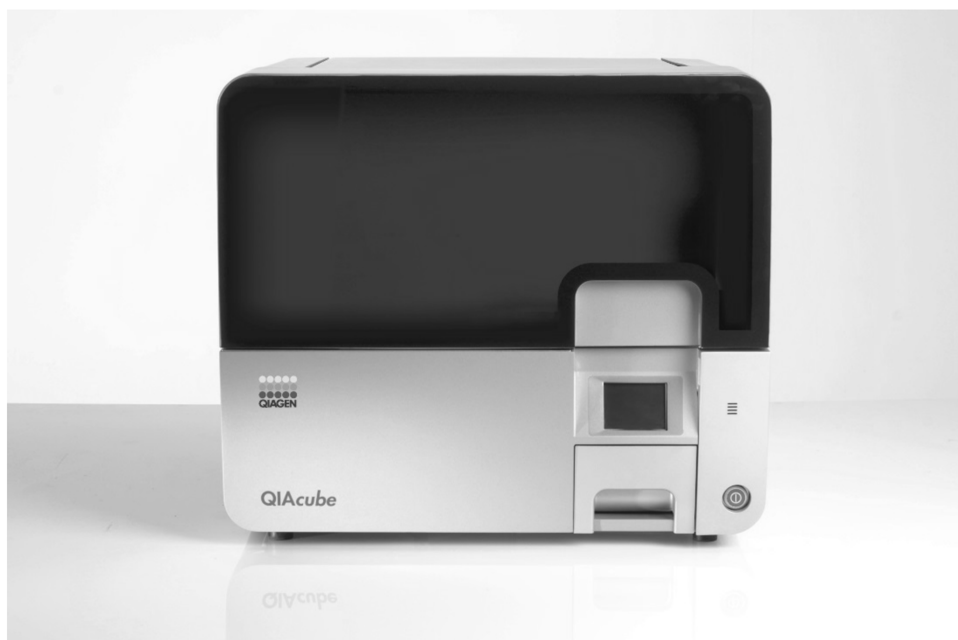


Figure 1. The QIAcube instrument.

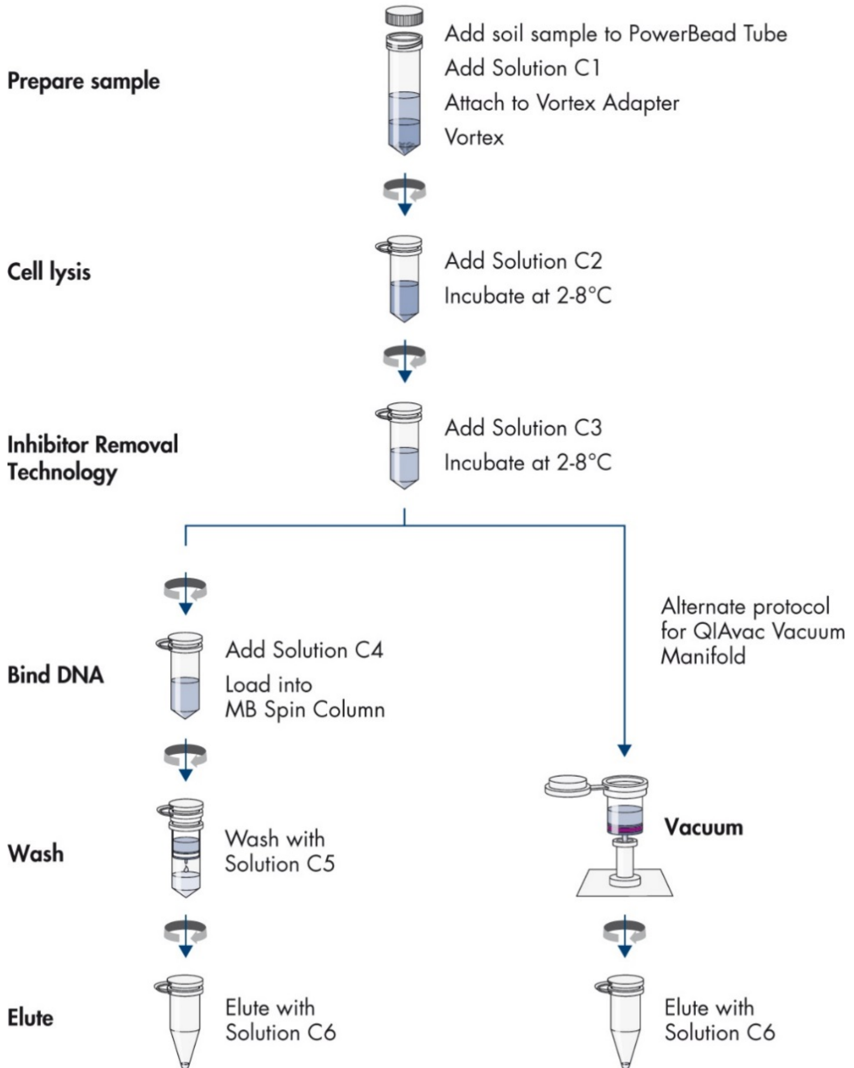


Figure 2. DNeasy PowerSoil Kit procedure.

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 (10,000 x *g*)
- Pipettors (50 µl–500 µl)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for 24 (1.5-2.0 ml) tubes (cat. no. 13000-V1-24)
- 100% ethanol (for the QIAvac 24 Plus Manifold protocol only)
- QIAvac 24 Plus Manifold

Important Notes

- Make sure the 2 ml PowerBead Tubes rotate freely in your centrifuge without rubbing.
- Shake to mix Solution C4 before use.

Protocol: Experienced User

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- 2 ml Collection Tubes are provided.

Procedure

1. Add 0.25 g of soil sample to the PowerBead Tube provided. Gently vortex to mix.
2. Add 60 µl of Solution C1 and invert several times or vortex briefly.

   Note: Solution C1 may be added to the PowerBead tube before adding soil sample
3. Secure PowerBead Tubes horizontally using a Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24).
4. Vortex at maximum speed for 10 min.

   Note: If using the 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.
5. Centrifuge tubes at 10,000 x *g* for 30 s.
6. Transfer the supernatant to a clean 2 ml Collection Tube.

   Note: Expect between 400–500 µl of supernatant. Supernatant may still contain some soil particles.
7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.

   Note: You can skip the 5 min incubation. However, if you have already validated the DNeasy PowerSoil extractions with this incubation we recommend you retain the step.
8. Centrifuge the tubes for 1 min at 10,000 x *g*.
9. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube.
10. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.

Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with this incubation we recommend you retain the step.

11. Centrifuge the tubes for 1 min at 10,000 x *g*.
12. Avoiding the pellet, transfer up to 750 µl of supernatant to a clean 2 ml Collection Tube.
13. Shake to mix Solution C4 and add 1200 µl to the supernatant. Vortex for 5 s.
14. Load 675 µl onto an MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard flow-through.
15. Repeat step 14 twice, until all of the sample has been processed.
16. Add 500 µl of Solution C5. Centrifuge for 30 s at 10,000 x *g*.
17. Discard the flow-through. Centrifuge again for 1 min at 10,000 x *g*.
18. Carefully place the MB Spin Column into a clean 2 ml Collection Tube. Avoid splashing any Solution C5 onto the column.
19. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you can use sterile DNA-free PCR-grade water for this step (cat. no. 17000-10).
20. Centrifuge at room temperature for 30 s at 10,000 x *g*. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

Protocol: Detailed

Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- 2 ml Collection Tubes are provided.

Procedure

1. Add 0.25 g of soil sample to the PowerBead Tube provided. Gently vortex to mix.
   Note: After your sample has been loaded into the PowerBead Tube, the next step is a homogenization and lysis procedure. The PowerBead 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 Tube and begins to disperse the sample in the buffer.
2. If Solution C1 has precipitated, heat at 60°C until precipitate dissolves. Add 60 µl of Solution C1 to sample and invert several times or vortex briefly.
   Note: Solution C1 may be added to the PowerBead tube before adding soil sample. Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS but will not harm it or the other disruption agents. Solution C1 can be used while it is still warm.
3. Secure PowerBead Tubes horizontally using a Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24).
4. Vortex at maximum speed for 10 min.
   Note: If using the 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min. Vortexing is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1–4 and mechanical shaking

introduced at this step. By randomly shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open. Use of 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.

5. Centrifuge tubes at 10,000 x *g* for 30 s.
6. Transfer the supernatant to a clean 2 ml Collection Tube.
   Note: Expect between 400–500 µl of supernatant. Supernatant may still contain some soil particles.
7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.
   Note: You can skip the 5 min incubation. However, if you have already validated the DNeasy PowerSoil extractions with this incubation we recommend you retain the step.
   Solution C2 is patented 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.
8. Centrifuge the tubes at 10,000 x *g* for 1 min.
9. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube.
   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.
10. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.
    Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with this incubation we recommend you retain the step.
    Solution C3 is patented Inhibitor Removal Technology (IRT) and is a second reagent to precipitate additional non-DNA organic and inorganic material including humic acid, cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.
11. Centrifuge the tubes at 10,000 x *g* for 1 min.

12. Avoiding the pellet, transfer up to 750 µl of supernatant to a clean 2 ml Collection Tube.
    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.
13. Shake to mix Solution C4 and add 1200 µl to the supernatant. Vortex for 5 s.
    Note: Solution C4 is a high-concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations 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 Columns.
14. Load 675 µl onto an MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard flow-through.
    Note: DNA is selectively bound to the silica membrane in the MB Spin Column device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.
15. Repeat step 14 twice, until all of the sample has been processed.
16. Add 500 µl of Solution C5. Centrifuge for 30 s at 10,000 x *g*.
    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.
17. Discard the-flow through. Centrifuge again for 1 min at 10,000 x *g*.
    Note: This flow-through fraction is non-DNA organic and inorganic waste removed from the silica MB Spin Column membrane by the ethanol wash solution. The second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications such as PCR, restriction digests and gel electrophoresis.
18. Carefully place the MB Spin Column into a clean 2 ml Collection Tube. Avoid splashing any Solution C5 onto the column.

19. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you can use sterile DNA-free PCR-grade water for this step (cat. no. 17000-10).
    Note: Placing the 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 silica MB Spin Column 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.
20. Centrifuge at room temperature for 30 s at 10,000 x *g*. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

Protocol: QIAvac 24 Plus Vacuum Manifold

Notes before starting

- For each sample lysate, use one MB Spin Column. Keep the MB Spin Column in the attached 2 ml Collection Tube and continue using the Collection Tube as an MB Spin Column holder until needed for the vacuum manifold protocol.
- Label each Collection Tube top and MB Spin Column to maintain sample identity. If the MB Spin Column becomes clogged during the vacuum procedure, switch to the centrifugation protocol.
- 100% ethanol will be needed for step 8 of this protocol.

Procedure

1. Connect the QIAvac 24 Plus to the vacuum source using the QIAvac Connecting System (for more details, refer to the QIAvac 24 Plus Handbook, Appendix A, page 16).
2. Insert a VacValve into each Luer slot of the QIAvac 24 Plus that is to be used. Close unused Luer slots with Luer plugs or close the inserted VacValve.
3. Insert a VacConnector into each VacValve. Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.
4. Place an MB Spin Column into each VacConnector on the manifold.
5. Transfer 650 µl of prepared sample lysate (from step 13 of the centrifugation protocol) to an MB Spin Column.
6. Turn on the vacuum source and open the VacValve of the port. Hold the tube in place when opening the VacValve to keep the MB Spin Column steady. Allow the lysate to pass through the MB Spin Column completely.
7. After the lysate has passed through the column completely, load again with 650 µl of lysate. Continue until all the lysate has been loaded onto the MB Spin Column. Close the VacValve of that port.

Note: If the MB Spin Columns are filtering slowly, close the ports to samples that have completed filtering to increase the pressure to the other columns.

8. Add 800 µl of 100% ethanol to completely fill the MB Spin Column. Open the VacValve while holding the column steady. Allow the ethanol to pass through the column completely. Close the VacValve.
9. Add 500 µl of Solution C5 to each MB Spin Column. Open the VacValve and apply a vacuum until Solution C5 has passed through the MB Spin Column completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
10. Turn off the vacuum source and open an unused port to vent the manifold. If all 24 ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.
11. Remove the MB Spin Column and place in the original labeled 2 ml Collection Tube. Place into the centrifuge and spin at 13,000 x *g* for 2 min to completely dry the membrane.
12. Transfer the MB Spin Column into a new 2 ml Collection Tube and add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-free PCR-grade water (cat. no. 17000-10) may be used.
13. Centrifuge at 13,000 x *g* for 1 min at room temperature.
14. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA, see 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 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 PowerBead Tube (beads and solution) and transfer into another sterile microcentrifuge tube (not provided). Add soil sample to PowerBead Tube and centrifuge at room temperature for 30 seconds at 10,000 x *g*. Remove as much liquid as possible with a pipet tip. Add beads and bead solution back to PowerBead Tube, gently vortex to mix 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 DNA Kit; however, it should still be attempted. If DNA will still not amplify after trying the steps above, then PCR optimization (changing reaction conditions and primer choice) 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 100 µl. The DNA may be concentrated by adding 4 µl of 5 M NaCl and inverting 3–5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3–5 times to mix. Centrifuge at 10,000 x *g* for 5 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac, a dessicator or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.

		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 19 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 C6 (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 with sterile DNA-free PCR grade water (cat. no. 17000-10).

Alternative lysis methods

a)	Cells are difficult to lyse	After adding Solution C1, incubate at 70°C for10 minutes. Resume protocol from step 3.
b)	Reduction of shearing of DNA	After adding Solution C1, vortex 3–4 seconds, then heat to 70°C for 5 minutes. Vortex 3–4 seconds. Heat another 5 minutes. Vortex 3–4 seconds. This alternative procedure will reduce shearing but may also reduce yield.

Ordering Information

Product	Contents	Cat. no.
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 PowerSoil HTP 96 Kit (384)	For 4 x 96 preps: High-throughput isolation of DNA from soil samples in less than one day	12955-4
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
DNeasy PowerLyzer PowerSoil Kit (50)	For 50 preps: Isolate DNA from tough soil microbes; optimized for use with bead-based homogenizers	12855-50
DNeasy PowerLyzer PowerSoil Kit (100)	For 100 preps: Isolate DNA from tough soil microbes; optimized for use with bead-based homogenizers	12855-100
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
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

Product	Contents	Cat. no.
Ceramic Bead Tubes, 1.4 mm (50)	Ready-to-use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials	13113-50
Glass Bead Tubes, 0.5 mm (50)	Ready-to-use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials	13116-50
Glass Bead Tubes, 0.1 mm (50)	Ready-to-use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials	13118-50
QIAvac 24 Plus Manifold	Vacuum Manifold for processing 1–24 spin columns; includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, and Quick Couplings	19413

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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