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DNeasy® PowerMax® Soil Kit Handbook

For the isolation of microbial DNA from large quantities of soil – great for samples with low microbial load

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy PowerMax Soil Kit	**(10)**
Catalog no.	**12988-10**
Number of preps	**10**
MB Maxi Spin Columns	10
PowerMax Bead Tubes	10
PowerBead Solution	200 ml
Solution C1	2 x 6.6 ml
Solution C2	2 x 28 ml
Solution C3	44 ml
Solution C4	330 ml
Solution C5	4 x 30 ml
Solution C6	66 ml
Collection Tubes (50 ml)	5 x 8
Quick Start Protocol	1

Storage

The DNeasy PowerMax Soil 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 and is flammable.**

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

PowerBead Solution 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 PowerMax Soil Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerMax Soil Kit comprises a novel and proprietary method for isolating genomic DNA from environmental samples using Inhibitor Removal Technology® (IRT). With this kit, it is possible to process samples that have proven difficult in the past due to high levels of humic-like substances. The isolated DNA has a high level of purity, which allows for successful PCR amplification from samples. Total DNA isolated from various soil types has been successfully amplified using PCR with primers specific for bacteria (*Bacillus subtilis*, *Bacillus anthracis*), fungi (yeast, mold) and actinomycetes (*Streptomyces*).

Using the DNeasy PowerMax Soil Kit, environmental samples are added to a bead beating tube with a kit-supplied proprietary buffer for rapid and thorough homogenization. Cell lysis and DNA exposure occurs by mechanical and chemical methods. Extracted genomic DNA is captured on a silica membrane in a spin column format. The DNA is washed and eluted from the membrane and is ready for PCR and other downstream applications.

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.

- Centrifuge capable of spinning 50 ml tubes at 2500 x *g* using swing-out rotor (please see additional information below)
- Pipettes (1 ml and 10 ml)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for 2 (50 ml) tubes (cat. no. 13000-V1-50)

Centrifugation

Centrifugation of MB Maxi Spin Columns is performed in 50 ml centrifuge tubes at 2500 x g. Please ensure that your centrifuge is equipped with a swing-out rotor able to reach the centrifugal force. Do not use a fixed-angle rotor. All centrifugation steps are carried out at room temperature (15–25°C).

Note: When placing the centrifuge tubes into the rotor buckets, make sure that lids of the tubes cannot touch each other during centrifugation. Failure to do so may cause tubes to break.

Note: Ensure that centrifuge tubes used can withstand the centrifugal forces required.

Protocol: Experienced User

Important points before starting

- Shake to mix Solution C4 before use.
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Please wear gloves at all times.

Procedure

1. Add 15 ml of PowerBead Solution to a PowerMax Bead Tube.
2. Add up to 10 g of soil sample to the PowerMax Bead Tube containing PowerBead Solution. Vortex vigorously for 1 minute.
   Note: Refer to the Troubleshooting Guide before deciding on the amount of soil to process.
3. Add 1.2 ml of Solution C1 to the PowerMax Bead Tube and vortex vigorously for 30 seconds.
4. Place the PowerMax Bead Tube on a vortex adapter (cat. no. 13000-V1-50) and vortex for 10 minutes at the highest speed. Alternatively, place the tube in a shaking water bath set at 65°C and shake at maximum speed for 30 minutes.
5. Centrifuge at 2500 x *g* for 3 minutes at room temperature.
6. Transfer supernatant to a clean Collection Tube (provided).
   Note: The supernatant may still contain some soil particles and color.
7. Add 5 ml of Solution C2. Invert twice to mix. Incubate at 2–8°C for 10 minutes.
8. Centrifuge at 2500 x *g* for 4 minutes at room temperature.
9. Avoiding the pellet, transfer supernatant to a clean Collection Tube (provided).
10. Add 4 ml of Solution C3 and invert twice to mix. Incubate at 2–8°C for 10 minutes.
11. Centrifuge at 2500 x *g* for 4 minutes at room temperature.
12. Avoiding the pellet, transfer supernatant to a clean Collection Tube (provided).

13. Shake to mix Solution C4. Add 30 ml of Solution C4 to supernatant and invert twice.
14. Fill an MB Maxi Spin Column with the solution from Step 13.
15. Centrifuge at 2500 x *g* for 2 minutes at room temperature. Discard the flow-through and add a second volume of supernatant to the same MB Maxi Spin Column and centrifuge again at 2500 x *g* for 2 minutes at room temperature. Discard the flow-through. Repeat until the entire volume has been processed. This will take up to 4 total spins.
16. Add 10 ml of Solution C5. Centrifuge at 2500 x *g* for 3 minutes at room temperature. Discard the flow-through.
17. Centrifuge at 2500 x *g* for 5 minutes at room temperature.
18. Carefully place the MB Maxi Spin Column in a new Collection Tube (provided). Avoid splashing Solution C5 onto the column.
19. Add 5 ml of sterile Solution C6 to the center of MB Maxi Spin Column membrane and centrifuge at 2500 x *g* for 3 minutes at room temperature.
20. 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.

Protocol: Detailed

Important points before starting

- Shake to mix Solution C4 before use.
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Please wear gloves at all times.

Procedure

1. Add 15 ml of PowerBead Solution to a PowerMax Bead Tube.
2. Add up to 10 g of soil sample to the PowerMax Bead Tube containing PowerBead Solution. Vortex vigorously for 1 minute.
   Note: Please refer to the Troubleshooting Guide before deciding on the amount of soil to process. After your sample has been loaded into the PowerMax Bead Tube, the next step is homogenization and lysis. The PowerMax Bead 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. Vortexing mixes the components in the PowerMax Bead Tube and begins to disperse the sample in the solution.
3. Add 1.2 ml of Solution C1 to the PowerMax Bead Tube and vortex vigorously for 30 seconds.
   Note: Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms.
4. Place the PowerMax Bead Tube on a vortex adapter (cat. no. 13000-V1-50) and vortex for 10 minutes at the highest speed. Alternatively, place the tube in a shaking water bath set at 65°C and shake at maximum speed for 30 minutes.
   Note: Vortexing is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1–2 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 at 2500 x *g* for 3 minutes at room temperature.
6. Transfer supernatant to a clean Collection Tube (provided).
   Note: The supernatant may still contain some soil particles and color. The presence of carry-over soil or a dark color in the mixture is expected for many soil types at this step. Subsequent steps in the protocol will remove both carry-over soil and coloration.
7. Add 5 ml of Solution C2. Invert twice to mix. Incubate at 2–8°C for 10 minutes.
   Note: Solution C2 is patented 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 at 2500 x *g* for 4 minutes at room temperature.
9. Avoiding the pellet, transfer the supernatant to a clean Collection Tube (provided).
   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 4 ml of Solution C3 and invert twice to mix. Incubate at 2–8°C for 10 minutes.
    Note: Solution C3 has 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 2500 x *g* for 4 minutes at room temperature.
12. Avoiding the pellet, transfer supernatant to a clean Collection Tube (provided).
    Note: The pellet contains additional non-DNA organic and inorganic material including humic acid, cell debris and proteins. For the best DNA yield and quality, avoid transferring any of the pellet.

13. Shake to mix Solution C4. Add 30 ml of Solution C4 to supernatant and invert twice.
    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. Fill an MB Maxi Spin Column with the solution from Step 13.
15. Centrifuge at 2500 x *g* for 2 minutes at room temperature. Discard the flow-through and add a second volume of supernatant to the same MB Maxi Spin Column and centrifuge again at 2500 x *g* for 2 minutes at room temperature. Discard the flow-through. Repeat until entire volume has been processed. This will take up to 4 total spins.
    Note: DNA is selectively bound to the silica membrane in the MB Maxi Spin Column device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.
16. Add 10 ml of Solution C5. Centrifuge at 2500 x *g* for 3 minutes at room temperature. Discard the flow-through.
    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 Maxi 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. Centrifuge at 2500 x *g* for 5 minutes at room temperature.
    Note: 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 Maxi Spin Column in a new Collection Tube (provided). Avoid splashing Solution C5 onto the column.
19. Add 5 ml of sterile Solution C6 to the center of MB Maxi Spin Column membrane and centrifuge at 2500 x *g* for 3 minutes at room temperature.
    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 Maxi 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. Alternatively, you can use sterile DNA-free PCR-grade water for this step (cat. no. 17000-10).

20. Discard the MB Maxi 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.

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 amount of soil to process will depend on the soil type. The typical amount recommended is 5 g, although dry soils may require less starting material (1 g) and wet soils may require more (up to 10 g). For mulch and potting mixtures, we recommend up to 2.5 g and for composts up to 5.0 g. Up to 10 g of sandy soil may be processed.
DNA		
a)	DNA does not amplify	Check DNA yield by gel electrophoresis and spectrophotometer reading. Template is typically added to 10 ng per reaction, although more or less may be needed depending on the reaction conditions, enzyme activity and copy number of the target sequence. If DNA will does not amplify after altering the amount of template in the reaction, then PCR optimization (changing reaction conditions and primer choice) may be needed.
b)	Concentrating eluted DNA	The final volume of eluted DNA will be 5 ml. The DNA may be concentrated by adding 0.2 ml of 5 M NaCl and inverting 3–5 times to mix. Next, add 10.4 ml of 100% cold ethanol and invert 3–5 times to mix. Centrifuge at 2500 x *g* for 5 minutes at room temperature. Decant all liquid (If sterile DNA is desired, wash the DNA pellet with 70% cold ethanol. Be sure not to disturb the pellet.) Remove residual ethanol in a speed vac, a dessicator or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.
c)	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 16 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.

		Comments and suggestions
d)	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 and the stored at –70°C.

Ordering Information

Product	Contents	Cat. no.
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 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 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
Related Products		
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 1.7 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24

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.

Revision History

Document Revision History	
R2 11/2018	Added Important Note on centrifugation.

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