January 2020

# DNeasy<sup>®</sup> PowerLyzer<sup>®</sup> PowerSoil<sup>®</sup> Kit Handbook

For the isolation of DNA from tough soil microbes; optimized for use with bead-based homogenizers



Sample to Insight

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### Kit Contents

DNeasy PowerLyzer PowerSoil Kit	(50)	(100)
Catalog no.	12855-50	12855-100
Number of preps	50	100
MB Spin Columns	50	2 x 50
PowerBead Tubes, Glass 0.1 mm	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 × 50
Quick-Start Protocol	1	1

### Storage

The DNeasy PowerLyzer 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.

QIAcube<sup>®</sup> 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.

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.



DO NOT add bleach or acidic solutions to 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 PowerLyzer PowerSoil Kits is tested against predetermined specifications to ensure consistent product quality.

### Introduction

The DNeasy PowerLyzer PowerSoil Kit differs from the DNeasy PowerSoil Kit because it includes PowerBead Tubes with glass beads that are optimized for robust bead-based homogenizers like the PowerLyzer 24 Homogenizer (110/220V) (cat. no. 13155) as well as Fast Prep<sup>®</sup> and Precellys<sup>®</sup> instruments.

### Principle and procedure

The DNeasy PowerLyzer PowerSoil Kit comprises a novel and proprietary method for isolating genomic DNA from environmental samples in a fraction of the time required by traditional homogenization methods. The kit utilizes Inhibitor Removal Technology<sup>®</sup> (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 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 used 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*). Homogenization with the PowerLyzer 24 Homogenizer is faster than using traditional vortex methods and minimizes cross-contamination.

The DNeasy PowerLyzer 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 and homogenized rapidly and thoroughly using the PowerLyzer 24 Homogenizer. Cell lysis occurs by mechanical and chemical interaction. 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.

#### Optimized for homogenization with the PowerLyzer 24 Homogenizer

The DNeasy PowerLyzer PowerSoil Kit contains PowerBead Tubes with 0.1 mm glass beads, which allows for more options in choosing homogenization methods, including the use of the PowerLyzer 24 Homogenizer. The PowerLyzer's velocity and proprietary motion combine to provide the fastest homogenization possible, minimizing time spent processing samples. The 0.1 mm PowerBead Tubes are suitable for both high-velocity bead beating and vortex beating, depending on the level of homogenization desired. Alternative pre-filled bead tube options are available using harder matrices for grinding.

For the PowerLyzer 24 Homogenizer, the starting point for low-biomass and clay soils is 45 seconds at a setting of 4000 RPM. For loamy soils, such as forest soils, settings between 2500–2800 RPM provide the highest yields without compromising integrity. To use the PowerLyzer 24 Homogenizer in place of a vortex, a setting of 2000 RPM may be used for up to 5 minutes. Settings and duration of homogenization may need to be optimized for specific soil types and research projects to ensure highest yields and integrity.

#### Using the DNeasy PowerLyzer PowerSoil Kit with other homogenizers

Published references for using the DNeasy PowerLyzer PowerSoil Kit with a FastPrep instrument are available from technical support. For more information, please contact Technical Support at **support.qiagen.com**.

To isolate DNA using the DNeasy PowerLyzer PowerSoil Kit with FastPrep or Precellys homogenizers, use the conversion chart (see Table 1 below) 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.

To start homogenizing, use a setting of 5 on the FastPrep or 5000 RPM on the Precellys for one pulse of 45 seconds using the PowerBead Tubes provided in this kit. For fungi or other species that are difficult to lyse, a 10-minute heating step at 65°C may be performed prior to bead beating. More than one pulse of bead beating or harder beads may be used. However, keep in mind that can cause DNA integrity to decrease.

PowerLyzer 24	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	-

Table 1. Conversion chart for using other homogenizers with the DNeasy PowerLyzer PowerSoil Kit

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

#### High-throughput options

We offer a vacuum-based protocol for faster processing without centrifugation for the DNAbinding and column-washing steps using MB Spin Columns. The QIAvac 24 Plus Manifold allows for processing of up to 24 MB Spin Column preps at a time. For additional highthroughput options, we offer the DNeasy 96 PowerSoil Pro (cat. no. 47017) for processing up to 2 x 96 samples using a centrifuge capable of spinning two stacked 96-well blocks (13 cm x 8.5 cm x 8 cm) at 4500 x g. For 96-well homogenization of soil, we offer the TissueLyser II and Plate Adapter Set (cat. no. 85300 and 11990, respectively.)

#### Automated purification of DNA on QIAcube Instruments

Purification of DNA 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 PowerLyzer PowerSoil Kit for purification of high-quality DNA.

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**.



QIAcube Connect.



#### DNeasy PowerLyzer 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.

- PowerLyzer 24 Homogenizer or another bead homogenizer
- Microcentrifuge (10,000 x g)
- Pipettors (60–750 µl)
- Vortex-Genie<sup>®</sup> 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)

### Important Notes

- Make sure the tubes rotate freely in your centrifuge without rubbing.
- The PowerLyzer 24 may cause marring of labels on the tops of the PowerBead Tubes. To ensure proper sample identification, label sides and tops of the tubes.

### 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.
- Shake to mix Solution C4 before use.

#### Procedure

- 1. Add up to 0.25 g of soil sample to the PowerBead Tube provided.
- 2. Add 750 µl of PowerBead Solution to the PowerBead Tube.
- 3. Add 60 µl of Solution C1 and invert several times or vortex briefly.
- 4. Bead beating options:

A. PowerLyzer 24 Homogenizer: Place the PowerBead Tubes into the tube holder for the PowerLyzer 24 Homogenizer. The PowerBead Tubes must be balanced in the tube holder. Run the samples for a time and RPM suitable for your soil type.
Note: For clay soils, 4,000 RPM for 45 s is the best starting point. For loose, granular and high organic soils, 2,500 RPM for 45 s will provide an optimal result.

**B.** Vortex: Secure the PowerBead Tubes horizontally using a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.

**Note:** If you are using a 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.

- Make sure the PowerBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge at 10,000 x g for 30 s. Do not exceed 10,000 x g.
   Note: Centrifuge for 3 min at 10,000 x g for clay soils or if your soil is not completely pelleted after 30 s.
- Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 400–500 μl. Supernatant may still contain some soil particles.

- 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 PowerSoil extractions with the incubation we recommend you retain the step.
- 8. Centrifuge the tubes for 1 min at 10,000 x g. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube (provided).
- 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 the incubation we recommend you retain the step.
- 10. Centrifuge the tubes for 1 min at 10,000 x g. Avoiding the pellet, transfer up to 750 µl of supernatant into a clean 2 ml Collection Tube (provided).
- 11. Add 1200  $\mu l$  of Solution C4 to the supernatant and vortex for 5 s.
- 12. Load 675  $\mu$ l of the supernatant onto an MB Spin Column and centrifuge at 10,000 x g for 1 min. Discard the flow-through and add an additional 675  $\mu$ l of supernatant.
- Centrifuge at 10,000 x g for 1 minute. Load the remaining supernatant onto the MB Spin Column and centrifuge at 10,000 x g for 1 min.
   Note: A total of three loads for each sample processed is required.
- 14. Add 500 µl of Solution C5 and centrifuge for 30 s at 10,000 x g.
- 15. Discard the flow-through. Centrifuge again for 1 min at  $10,000 \times g$ .
- 16. Carefully place the MB Spin Column in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the MB Spin Column.
- 17. Add 100 μl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile DNA-free PCR-grade water (cat. no. 17000-10) or TE buffer.
- 18. Centrifuge 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 (-90°C to -15°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

### Protocol: Detailed

#### 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.
- Shake to mix Solution C4 before use.

#### Procedure

- Add up to 0.25 g of soil sample to the PowerBead Tube provided.
   Note: Once the sample is loaded into a PowerBead Tube, the next step is homogenization and lysis. The PowerBead Tube will help disperse the soil particles.
- 2. Add 750 µl of PowerBead Solution to the PowerBead Tube.
- Add 60 µl of Solution C1 and invert several times or vortex briefly.
   Note: Vortexing mixes the components in the PowerBead Tube and begins to disperse the sample.
- 4. Bead beating options:

A. PowerLyzer 24 homogenizer: Place the PowerBead Tubes into the tube holder for the PowerLyzer 24 Homogenizer. The PowerBead Tubes must be balanced in the tube holder. Run the samples for a time and RPM suitable for your soil type.
Note: For clay soils, 4,000 RPM for 45 s is the best starting point. For loose, granular and high organic soils, 2,500 RPM for 45 s will provide an optimal result.

**B.** Vortex: Secure the PowerBead Tubes horizontally using a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.

**Note:** If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min. The bead beating or vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1–3 and mechanical shaking introduced at this step. By shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.

**Note:** The PowerLyzer 24 can homogenize soils at high acceleration in only 45 s, using the glass beads to achieve lysis in less time. The time and speed for each soil may vary. PowerBead Tubes may also be used with a Vortex-Genie 2 and a Vortex Adapter (cat. no. 13000-V1-24). The Vortex Adapter is designed to be a simple platform to keep the tubes tightly attached to the vortex. Using tape to attach tubes is not recommended.

- Make sure the PowerBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge at 10,000 x g for 30 s. Do not exceed 10,000 x g.
   Note: Centrifuge for 3 min at 10,000 x g for clay soils or if your soil is not completely pelleted after 30 s.
- Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 400–500 μl. 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 PowerSoil extractions with the incubation we recommend you retain the step. Solution C2 has 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.

- Centrifuge the tubes for 1 min at 10,000 x g. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml 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.
- 9. 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 the incubation we recommend you retain the step. Solution C3 has 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.
- Centrifuge the tubes for 1 min at 10,000 x g. Avoiding the pellet, transfer up to 750 µl of supernatant into a clean 2 ml 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.
- 11. Add 1200 µl of Solution C4 to the supernatant and 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.
- 12. Load 675  $\mu$ l of the supernatant onto a MB Spin Column and centrifuge at 10,000 x g for 1 min. Discard the flow-through and add an additional 675  $\mu$ l of supernatant.
- 13. Centrifuge at 10,000 x g for 1 minute. Load the remaining supernatant onto the MB Spin Column and centrifuge at 10,000 x g for 1 min.

**Note:** A total of three loads for each sample processed is required. 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.

- 14. Add 500 µl of Solution C5 and 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.
- 15. 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.
- 16. Carefully place spin filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the MB Spin Column.
- 17. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile DNA-free PCR-grade water (cat. no. 17000-10) or TE buffer. Note: Placing Solution C6 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 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.
- 18. Centrifuge 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 (-90°C to -15°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

### Protocol: QIAvac 24 Plus Vacuum Manifold

#### Important points before starting

- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution C4 before use.
- 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 (after step 11 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.

 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.

- 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.
- 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 the 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.
- 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.
- 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 ( $15-25^{\circ}$ C).
- 14. Discard the MB Spin Column. The DNA is now ready for downstream applications. Note: We recommend storing DNA frozen (–90°C to –15°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**).

Soil	Soil processing		
a)	Amount of soil to process	The QIAGEN DNeasy PowerLyzer 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	Add soil sample to PowerBead Tube and centrifuge at 10,000 x g for 30 seconds at room temperature (15–25°C). Remove as much liquid as possible with a pipet tip. Resume protocol from Step 2.	
DN/	A		
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 PowerLyzer 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.	

#### **Comments and suggestions**

#### Comments and suggestions

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 $\mu$ l. The DNA may be concentrated by adding 10 $\mu$ l of 5 M NaCl and inverting 3–5 times to mix. Next, add 200 $\mu$ l of 100% cold ethanol and invert 3–5 times to mix. Centrifuge at 10,000 x g for 5 minutes at room temperature (15–25°C). Decant all liquid. Remove residual ethanol in a speed vac, a desiccator or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.
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 -90°C to -15°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).
Alte	rnative 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 PowerLyzer PowerSoil Kit (50)	For the bead-based isolation of DNA from tough soil microbes	12855-50
DNeasy PowerLyzer PowerSoil Kit (100)	For the isolation of DNA from tough soil microbes, optimized for use with bead-based homogenizers	12855-100
DNeasy PowerSoil Pro Kit (50)	For the isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For the isolation of microbial genomic DNA from all soil types	47016
DNeasy 96 PowerSoil Pro Kit (384)	For the isolation of DNA from up to 384 soil samples in less than one day	47017
DNeasy PowerMax® Soil Kit (10)	For the isolation of microbial DNA from large quantities of soil with low microbial load	12988-10
DNeasy PowerClean® Cleanup Kit (50)	For secondary DNA clean-up and removal of inhibitors from heparin	12877-50
QIAcube Connect — fo QIAGEN spin-column	or fully automated nucleic acid extraction with kits	
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395

Product	Contents	Cat. no.
Related products		
RNeasy® PowerSoil Total RNA Kit (25)	For the isolation of high quality total RNA from all soil types	12866-25
MagAttract® PowerSoil DNA KF Kit (384)	For the automated, hands-free isolation of DNA from soil	270004-KF
Accessories		
PowerBead Tubes, Ceramic 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
PowerBead Tubes, Glass 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
PowerBead Tubes, Glass 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
Vortex Adapter	For vortexing 1.7 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
QIAvac 24 Plus	Vacuum Manifold for processing 1–24 spin columns; includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs and Quick Couplings	19413

\* 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
January 2020	Updated text, ordering information and intended use for QIAcube Connect.

Notes

#### Limited License Agreement for DNeasy PowerLyzer PowerSoil Kit

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

- 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.ajagen.com. Some of these additional protocols have been provided by 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.
- 2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
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