August 2019

DNeasy® PowerSoil® HTP 96 Kit Handbook

For high-throughput isolation of DNA from up to 384 soil samples

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy PowerSoil HTP 96 Kit (384) **Catalog no. 12955-4** **Number of preps 384**	
QIAamp® 96 Plates	4
PowerBead DNA Plates, Garnet	4
PowerBead Solution	2 x 200 ml
Solution C1	45 ml
Solution C2	128 ml
Solution C3	106 ml
Solution C4	2 x 330 ml
Solution C5-D*	120 ml
Solution C6	66 ml
Racked Elution Microtubes	4
Caps for Elution Microtubes	50 x 8
Collection Plates (1 ml)	4 x 4
Collection Plates (2 ml)	4
AirPore Tape Sheets	25
Sealing Tape, Polyester (2 ml)	16
S-Blocks	4
Square Well Mats	4
Quick Start Protocol	1

*Before using for the first time, add 100% ethanol to Solution C5-D, as indicated in the protocol, to obtain a working solution.

Storage

The DNeasy PowerSoil HTP 96 Kit reagents and components can be stored at room temperature (15–25°C) until the expiry 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-D is flammable after addition of ethanol.

WARNING

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 PowerSoil HTP 96 Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerSoil HTP 96 Kit allows high-throughput isolation of DNA from up to 384 soil samples in less than one day.

Two protocols are included for use with this product. The first protocol combines both vacuum and centrifugation steps. The second protocol uses centrifugation exclusively. This kit requires the use of a specialized plate shaker to facilitate the bead beating process in the PowerBead DNA Plates. We recommend the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990).

Principle and procedure

This kit provides researchers with a high-throughput method for isolating genomic DNA from environmental samples using Inhibitor Removal Technology® (IRT) that efficiently removes humic substances that inhibit PCR. This procedure effectively removes PCR inhibitors from even the most difficult soil types, allowing for more successful PCR amplification of DNA. DNA isolated from many sample types, including compost, sediment and manure, was successfully used as template to amplify members of a wide range of microbial groups in soils. These include bacteria (gram-positive, gram-negative and spore-formers), actinomycetes, archaebacteria and fungi.

Environmental samples are added to a 96 well bead beating plate for rapid and thorough homogenization. Cell lysis occurs by a combination of mechanical and chemical methods. Humic substances are removed by a specialized precipitation process. Total genomic DNA is captured on a 96 well silica membrane in a spin-column plate format. DNA is then washed and eluted from the membrane. The eluted DNA is ready for PCR analysis and other downstream applications.

The estimated time from start to finish to process two 96 well plates for this protocol is approximately 8 hours. Stopping points at appropriate steps are mentioned in the protocol. The majority of the time is for weighing and loading the soil samples into the 96 well plates.

Equipment and Reagents to Be Supplied by User

- Centrifuge capable of handling two 96 well blocks (13 cm x 8.5 cm x 60 cm) at 4500 x *g*

  Note: If you have a centrifuge with a maximum speed less than 4500 x *g*, see the Troubleshooting Guide.
- Multi-channel pipettor (50–650 µl)
- Mechanical shaker for 96 well blocks and plate adaptors (cat. no. 11990)
- Vortex-Genie® 2 vortex with 3-inch platform
- 100% ethanol
- Reagent reservoirs (optional)
- Vacuum pump (optional)
- Vacuum manifold (optional)
- Plate seals (optional – if protocol is paused at step 15)

Important Notes

Before using the kit for the first time: Prepare solution C5-D by adding an equal volume (120 ml) of 100% ethanol. Mix well.

Protocol: Experienced User, Centrifugation

Important points before starting

- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- To prepare Solution C5-D **before first use**, add equal volume (120 ml) of 100% ethanol. Mix well.
- Please wear gloves at all times.
- The assembled S-Block and QIAamp 96 Plate may not fit some centrifuge types. Please contact QIAGEN Technical Support at **support.qiagen.com** if the S-Block/QIAamp 96 Plate does not fit into your centrifuge buckets.

Procedure

1. Remove Square Well Mat from a PowerBead Plate. Add up to 0.25 g of soil sample.

   Note: Avoid cross contamination between sample wells. This is an appropriate stopping point. You can store the PowerBead Plate at 2–8°C covered with the Square Well Mat.
2. Add 750 µl of PowerBead Solution to the wells of the PowerBead Plate.
3. Add 60 µl of Solution C1. Secure the Square Well Mat tightly to the plate.
4. Place PowerBead Plate with mat securely fastened between 2 Adapter Plates (cat. no. 11990) on a 96 well plate shaker or a TissueLyser II (cat. no. 85300).
5. Shake at speed 20 Hz for 10 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 10 min.
6. Centrifuge at room temperature for 6 min at 4500 x *g*.
7. Discard the Square Well Mat. Transfer the supernatant to a clean 1 ml Collection Plate.

   Note: The supernatant may still contain some soil particles.
8. Add 250 µl of Solution C2.

9. Apply Sealing Tape to plate. Vortex for 5 s. Incubate at 2–8°C for 10 min.

   Note: You can skip the 10 min incubation. However, if you have already validated DNeasy PowerSoil extractions with the incubation, we recommend you retain the step.

10. Centrifuge the plate at room temperature for 6 min at 4500 x *g*. Discard Sealing Tape.
11. Avoiding the pellet, transfer entire volume of supernatant to a new 1 ml Collection Plate.
12. Apply Sealing Tape to plate and repeat steps 10–11 once. Then move on to step 13.
13. Add 200 µl of Solution C3 and repeat steps 9–11 once. Then apply Sealing Tape to the plate and centrifuge again at room temperature for 6 min at 4500 x *g*.
14. Transfer no more than 650 µl of supernatant to a 2 ml Collection Plate.
15. Add 650 µl of Solution C4 to each well of the plate. Repeat (to add 1300 µl total).

    Note: You can pause here and store the samples covered with Sealing Tape at 2–8°C.

16. Pipet samples up and down to mix. Place a spin plate onto an S-Block.
17. Load approximately 650 µl into each well of the spin plate and seal the plate with an AirPore Tape Sheet.
18. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the spin plate back on the same S-Block. Discard the AirPore Tape Sheet.
19. Repeat steps 17 and 18 until all the supernatant has been processed. Discard the final flow-through.
20. Place the spin plate back on the same S-Block.
21. Add 500 µl of Solution C5-D to each well of the spin plate and seal the plate with an AirPore Tape Sheet.
22. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the spin plate back on the same S-Block. Seal with an AirPore Tape Sheet.
23. Centrifuge again at room temperature for 5 min at 4500 x *g*. Discard flow-through.
24. Carefully place the spin plate onto Racked Elution Microtubes. Discard the AirPore Tape Sheet.
25. Allow to air dry for 10 min at room temperature.

26. Add 100 µl of Solution C6 to the center of each well. Seal plate with an AirPore Tape Sheet.
27. Centrifuge at 4500 x *g* for 3 min at room temperature. Discard the AirPore Tape Sheet.
28. Seal Elution Microtubes with the Caps provided. The DNA is now ready for downstream applications.

    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution C6 does not contain EDTA. To concentrate DNA, refer to the Troubleshooting Guide.

Protocol: Detailed, Centrifugation

Important points before starting

- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- To prepare Solution C5-D **before first use**, add equal volume (120 ml) of 100% ethanol. Mix well.
- Please wear gloves at all times.
- The assembled S-Block and QIAamp 96 Plate may not fit some centrifuge types. Please contact QIAGEN Technical Support at **support.qiagen.com** if the S-Block/QIAamp 96 Plate does not fit into your centrifuge buckets.

Procedure

1. Remove Square Well Mat from a PowerBead Plate. Add up to 0.25 g of soil sample.

   Note: Avoid cross contamination between sample wells. This is an appropriate stopping point. You can store the PowerBead Plate at 2–8°C covered with the Square Well Mat.

2. Add 750 µl of PowerBead Solution to the wells of the PowerBead Plate.

   Note: This is the first part of the lysis procedure. The PowerBead Solution is a buffer that will disperse the soil particles.

3. Add 60 µl of Solution C1. Secure the Square Well Mat tightly to the plate.

   Note: Solution C1 contains SDS, which is a detergent that aids in cell lysis. SDS breaks down fatty acids and lipids associated with the cell membrane of microorganisms. If it gets cold, SDS will precipitate. Heating at 60°C will dissolve the SDS. Solution C1 can be used while it is still warm.

4. Place PowerBead Plate with mat securely fastened between 2 Adapter Plates (cat. no. 11990) on a 96 well plate shaker or TissueLyser II (cat. no. 85300).

5. Shake at speed 20 Hz for 10 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 10 min.

   Note: This protocol uses a combination of mechanical and chemical lysis. Mechanical lysis is introduced at this step. By randomly shaking the beads, they collide with one another and with microbial cells causing them to break open.

6. Centrifuge at room temperature for 6 min at 4500 x *g*.

   Note: Particulates, including cell debris, soil, beads and humic acids, will form a pellet at this point. DNA is in the supernatant.

7. Discard the Square Well Mat. Transfer the supernatant to a clean 1 ml Collection Plate.

   Note: The supernatant may still contain some soil particles.

8. Add 250 µl of Solution C2.

   Note: Solution C2 contains a reagent to 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.

9. Apply Sealing Tape to plate. Vortex for 5 s. Incubate at 2–8°C for 10 min.

   Note: You can skip the 10 min incubation. However, if you have already validated DNeasy PowerSoil extractions with the incubation we recommend you retain the step.

10. Centrifuge the plate at room temperature for 6 min at 4500 x *g*. Discard Sealing Tape.
11. Avoiding the pellet, transfer entire volume of supernatant to a new 1 ml Collection Plate.
12. Apply Sealing Tape to plate and repeat steps 10–11 once. Then move on to step 13.
13. Add 200 µl of Solution C3 and repeat steps 9–11 once. Then apply Sealing Tape to the plate and centrifuge at room temperature for 6 min at 4500 x *g*.

    Note: Solution C3 is a second reagent to precipitate additional 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.

14. Transfer no more than 650 µl of supernatant to a 2 ml Collection Plate.

15. Add 650 µl of Solution C4 to each well of the plate. Repeat (to add 1300 µl total).

    Note: You can pause here and store the samples covered with Sealing Tape at 2–8°C.

    Note: Solution C4 is a high-concentration salt solution. DNA binds tightly to silica at high salt concentrations, and this solution will adjust the 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 spin plate.

16. Pipet samples up and down to mix. Place a spin plate onto an S-Block.
17. Load approximately 650 µl into each well of the spin plate and seal the plate with an AirPore Tape Sheet.
18. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the spin plate back on the same S-Block. Discard the AirPore Tape Sheet.
19. Repeat steps 17 and 18 until all the supernatant has been processed. Discard the final flow-through.

    Note: In the high-salt solution, DNA is selectively bound to the silica membrane in the spin plate. Contaminants pass through the silica membrane, leaving only DNA bound to the membrane.

20. Place the spin plate back on the same S-Block.
21. Add 500 µl of Solution C5-D to each well of the spin plate and seal the plate with an AirPore Tape Sheet.

    Note: Solution C5-D is an ethanol-based wash solution used to further clean the DNA bound to the silica membrane in the spin plate. This wash solution removes residues of salt, humic acid and other contaminants while allowing the DNA to stay bound to the silica membrane. The flow-through is waste containing ethanol wash solution and contaminants that did not bind to the silica membrane.

    Note: You can wash more than one time to further clean DNA if desired. In some cases where soils have very high humic acid content, it will be beneficial to repeat this wash step. There is 10% extra Solution C5-D in the bottle for this purpose. Solution C5-D is also sold separately (cat. no. 12955-4-5D).

22. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the spin plate back on the same S-Block. Seal with an AirPore Tape Sheet.

    Note: This step removes residual Solution C5-D. It is critical to remove all traces of wash solution because it can interfere with downstream DNA applications.

23. Centrifuge again at room temperature for 5 min at 4500 x *g*. Discard flow-through.

    Note: Once again it is important to avoid any traces of the Solution C5-D.

24. Carefully place the spin plate onto Racked Elution Microtubes. Discard the AirPore Tape Sheet.

25. Allow to air dry for 10 min at room temperature.

    Note: This step removes residual Solution C5-D.

26. Add 100 µl of Solution C6 to the center of each well. Seal plate with an AirPore Tape Sheet.

    Note: Placing Solution C6 (elution buffer) in the center of the membrane will make sure the entire membrane is wet. This will result in more efficient and complete release of the DNA from the silica spin plate membrane.

27. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the AirPore Tape Sheet.
    Note: As Solution C6, which is a low salt solution, passes through the silica membrane, DNA that was bound in the presence of high salt is now selectively released.

28. Seal Elution Microtubes with the Caps provided. The DNA is now ready for downstream applications.

    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution C6 does not contain EDTA. To concentrate DNA, refer to the Troubleshooting Guide.

Protocol: Experienced User, Centrifugation and Vacuum

Important points before starting

- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- To prepare Solution C5-D **before first use**, add equal volume (120 ml) of 100% ethanol. Mix well.
- Please wear gloves at all times.
- The assembled S-Block and QIAamp 96 Plate may not fit some centrifuge types. Please contact QIAGEN Technical Support at **support.qiagen.com** if the S-Block/QIAamp 96 Plate does not fit into your centrifuge buckets.

Procedure

1. Remove Square Well Mat from a PowerBead Plate. Add up to 0.25 g of soil sample.

   Note: Avoid cross contamination between sample wells. This is an appropriate stopping point. You can store the PowerBead Plate at 2–8°C covered with the Square Well Mat.
2. Add 750 µl of PowerBead Solution to the wells of the PowerBead Plate.
3. Add 60 µl of Solution C1. Secure the Square Well Mat tightly to the plate.
4. Place PowerBead Plate with mat securely fastened between 2 Adapter Plates (cat. no. 11990) on a 96 well plate shaker or TissueLyser II (cat. no. 85300).
5. Shake at speed 20 Hz for 10 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 10 min.
6. Centrifuge at room temperature for 6 min at 4500 x *g*.
7. Discard the Square Well Mat. Transfer the supernatant to a clean 1 ml Collection Plate.

   Note: The supernatant may still contain some soil particles.
8. Add 250 µl of Solution C2.

9. Apply Sealing Tape to plate. Vortex for 5 s. Incubate at 2–8°C for 10 min.

   Note: You can skip the 10 min incubation. However, if you have already validated DNeasy PowerSoil extractions with the incubation, we recommend you retain the step.

10. Centrifuge the plate at room temperature for 6 min at 4500 x *g*. Discard Sealing Tape.
11. Avoiding the pellet, transfer entire volume of supernatant to a new 1 ml Collection Plate.
12. Apply Sealing Tape to plate and repeat steps 10–11 once. Then move on to step 13.
13. Add 200 µl of Solution C3 and repeat steps 9–11 once. Then apply Sealing Tape to the plate and centrifuge at room temperature for 6 min at 4500 x *g*.
14. Transfer no more than 650 µl of supernatant to a 2 ml Collection Plate.
15. Add 650 µl of Solution C4 to each well of the plate. Repeat (to add 1300 µl total).

    Note: You can pause here and store the samples covered with Sealing Tape at 2–8°C.

16. Remove the top portion of a vacuum manifold and place a new 2 ml Collection Plate in the bottom of the vacuum manifold.
17. Replace the top of the manifold and place a spin plate on it. Turn the vacuum pump on.
    Note: Test that you have a good seal with the manifold and the spin plate. You should be able to gently lift the entire unit without the spin plate separating from the manifold.
18. Load 650 µl of the samples into the wells of the spin plate and allow the samples to flow through. Repeat until all the samples have been processed. Turn off the vacuum.
19. Remove the spin plate and set aside. Discard the flow-through from the 2 ml Collection Plate and then place it back into the manifold.
20. Replace the spin column on top of the manifold. Turn the vacuum on and test the seal.
21. Add 500 µl of Solution C5-D to each well of the spin plate. Once the entire volume of Solution C5-D has passed through the well, turn off the vacuum.
22. Place the spin plate on an S-Block. Seal the plate with an AirPore Tape Sheet.
23. Centrifuge at room temperature for 5 min at 4500 x *g*. Discard flow-through.
24. Carefully place the spin plate onto Racked Elution Microtubes. Discard the AirPore Tape Sheet.

25. Allow to air dry for 10 min at room temperature.
26. Add 100 µl of Solution C6 to the center of each well. Seal plate with an AirPore Tape Sheet.
27. Centrifuge at 4500 x *g* for 3 min at room temperature. Discard the AirPore Tape Sheet.
28. Seal Elution Microtubes with the Caps provided. The DNA is now ready for downstream applications.

    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution C6 does not contain EDTA. To concentrate DNA, refer to the Troubleshooting Guide.

Protocol: Detailed, Centrifugation and Vacuum

Important points before starting

- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- To prepare Solution C5-D **before first use**, add equal volume (120 ml) of 100% ethanol. Mix well.
- Please wear gloves at all times.
- The assembled S-Block and QIAamp 96 Plate may not fit some centrifuge types. Please contact QIAGEN Technical Support at **support.qiagen.com** if the S-Block/QIAamp 96 Plate does not fit into your centrifuge buckets.

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1. Remove Square Well Mat from a PowerBead Plate. Add up to 0.25 g of soil sample.

   Note: Avoid cross contamination between sample wells. This is an appropriate stopping point. You can store the PowerBead Plate at 2–8°C covered with the Square Well Mat.

2. Add 750 µl of PowerBead Solution to the wells of the PowerBead Plate.

   Note: This is the first part of the lysis procedure. The PowerBead Solution is a buffer that will disperse the soil particles.

3. Add 60 µl of Solution C1. Secure the Square Well Mat tightly to the plate.

   Note: Solution C1 contains SDS, which is a detergent that aids in cell lysis. SDS breaks down fatty acids and lipids associated with the cell membrane of microorganisms. If it gets cold, SDS will precipitate. Heating at 60°C will dissolve the SDS. Solution C1 can be used while it is still warm.

4. Place PowerBead Plate with mat securely fastened between 2 Adapter Plates (cat. no. 11990) on a 96 well plate shaker or TissueLyser II (cat. no. 85300).

5. Shake at speed 20 Hz for 10 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 10 min.

   Note: This protocol uses a combination of mechanical and chemical lysis. Mechanical lysis is introduced at this step. By randomly shaking the beads, they collide with one another and with microbial cells causing them to break open.

6. Centrifuge at room temperature for 6 min at 4500 x *g*.

   Note: Particulates, including cell debris, soil, beads and humic acids, will form a pellet at this point. DNA is in the supernatant.

7. Discard the Square Well Mat. Transfer the supernatant to a clean 1 ml Collection Plate.

   Note: The supernatant may still contain some soil particles.

8. Add 250 µl of Solution C2.

   Note: Solution C2 contains a reagent to 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.

9. Apply Sealing Tape to plate. Vortex for 5 s. Incubate at 2–8°C for 10 min.

   Note: You can skip the 10 min incubation. However, if you have already validated DNeasy PowerSoil extractions with the incubation we recommend you retain the step.

10. Centrifuge the plate at room temperature for 6 min at 4500 x *g*. Discard Sealing Tape.
11. Avoiding the pellet, transfer entire volume of supernatant to a new 1 ml Collection Plate.
12. Apply Sealing Tape to plate and repeat steps 10–11 once. Then move on to step 13.
13. Add 200 µl of Solution C3 and repeat steps 9–11 once. Then apply Sealing Tape to the plate and centrifuge at room temperature for 6 min at 4500 x *g*.

    Note: Solution C3 is a second reagent to precipitate additional 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.

14. Transfer no more than 650 µl of supernatant to a 2 ml Collection Plate.

15. Add 650 µl of Solution C4 to each well of the plate. Repeat (to add 1300 µl total).

    Note: You can pause here and store the samples covered with Sealing Tape at 2–8°C.

    Note: Solution C4 is a high-concentration salt solution. DNA binds tightly to silica at high salt concentrations, and this solution will adjust the 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 spin plate.
16. Remove the top portion of a vacuum manifold and place a new 2 ml Collection Plate in the bottom of the vacuum manifold.
17. Replace the top of the manifold and place a spin plate on it. Turn the vacuum pump on.

    Note: Test that you have a good seal with the manifold and the spin plate. You should be able to gently lift the entire unit without the spin plate separating from the manifold.
18. Load 650 µl of the samples into the wells of the spin plate and allow the samples to flow through. Repeat until all the samples have been processed. Turn off the vacuum.

    Note: In the high-salt solution, DNA is selectively bound to the silica membrane in the spin plate. Contaminants pass through the silica membrane, leaving only DNA bound to the membrane.
19. Remove the spin plate and set aside. Discard the flow-through from the 2 ml Collection Plate and then place it back into the manifold.
20. Replace the spin column on top of the manifold. Turn the vacuum on and test the seal.
21. Add 500 µl of Solution C5-D to each well of the spin plate. Once the entire volume of Solution C5-D has passed through the well, turn off the vacuum.

    Note: Solution C5-D is an ethanol-based wash solution used to further clean the DNA bound to the silica membrane in the spin plate. This wash solution removes residues of salt, humic acid and other contaminants while allowing the DNA to stay bound to the silica membrane. The flow-through is waste containing ethanol wash solution and contaminants that did not bind to the silica membrane.

    Note: You can wash more than one time to further clean DNA if desired. In some cases where soils have very high humic acid content, it will be beneficial to repeat this wash step. There is 10% extra Solution C5-D in the bottle for this purpose. Solution C5-D is also sold separately (cat. no. 12955-4-5D).

22. Place the spin plate on an S-Block. Seal the plate with an AirPore Tape Sheet.
23. Centrifuge at room temperature for 5 min at 4500 x *g*. Discard flow-through.

    Note: This step removes residual Solution C5-D. It is critical to remove all traces of wash solution because it can interfere with downstream DNA applications.
24. Carefully place the spin plate onto Racked Elution Microtubes. Discard the AirPore Tape Sheet.
25. Allow to air dry for 10 min at room temperature.
26. Add 100 µl of Solution C6 to the center of each well. Seal plate with an AirPore Tape Sheet.

    Note: Placing Solution C6 (elution buffer) in the center of the membrane will make sure the entire membrane is wet. This will result in more efficient and complete release of the DNA from the silica spin plate membrane.
27. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard AirPore Tape Sheet. Note: As Solution C6, which is a low salt solution, passes through the silica membrane, DNA that was bound in the presence of high salt is now selectively released.
28. Seal Elution Microtubes with the Caps provided. The DNA is now ready for downstream applications.

    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution C6 does not contain EDTA. To concentrate DNA, 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
Sample processing	
a) Soil sample is high in water content	Weigh the slurry and dispense into the wells. We suggest restricting the starting amount to 0.25 g. Increasing the amount used will increase the amount of volume in the subsequent steps.
b) A vacuum step seems to be taking a long time	Turn off the vacuum source and lift the filter plate off the vacuum to release any back pressure. Replace the filter plate and turn the vacuum back on. Be sure there are no air leaks around the plate. If slow vacuum continues, you can centrifuge the filter plate as an alternative. Make sure any unused wells are covered with Sealing Tape.
c) Centrifuge available has maximum speed less than 4500 x *g*	Multiply the protocol time and speed to determine total x *g*. Divide the total by the maximum speed of the centrifuge (round up if necessary). This will be the number of minutes the centrifuge will need to run to achieve the appropriate overall force. **Example**: 10 min at 4500 x *g* = 45000. If centrifuge has a maximum speed of 2500 x *g*: 45000 ÷ 2500 = 18 min of centrifugation.
DNA	
a) DNA does not PCR 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 does not amplify after altering the amount of template in the reaction, PCR optimization (i.e., changing reaction conditions, validating primers or testing a different polymerase) should be attempted.

b) Concentrating eluted DNA

The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 5 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. Incubate at –15 to –30°C for at least 10 minutes to overnight. Centrifuge for 5 minutes at 10,000 x *g* at room temperature. Decant all liquid. Wash the DNA pellet with 70% cold ethanol. Centrifuge at 13,000 x *g* for 10 minutes to re-pellet the sample. Decant ethanol and dry in a speed vacuum, desiccator, or ambient air. Resuspend precipitated DNA in sterile water or 10 mM Tris.

Note: This procedure must be done individually after transferring the eluted samples to microcentrifuge tubes.

c) DNA floats out of well when loading a gel

This usually occurs because residual Solution C5-D remains in the final sample. Prevent this by being careful not to transfer liquid onto the bottom of the spin plate. Ethanol precipitation (described in "Concentrating the DNA") is the best way to remove residual Solution C5-D.

d) Eluted DNA has color

If you are working with a soil sample that has an extremely high humic acid content, there is the unlikely possibility that your eluted solution may contain some color. If this occurs, increase the number of washes with Solution C5-D to two or three. If you are still encountering coloring, please contact QIAGEN Technical Support at **support.qiagen.com**.

e) Storing DNA

DNA is eluted in Solution C6 (10 mM Tris). Store the DNA at –15 to –30°C to prevent degradation. DNA can be eluted in TE without DNA 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). DNA that has been eluted into sterile water should be stored at –65 to –90°C. Prolonged storage in the microplates at 2–8°C will result in the loss of liquid due to evaporation.

Alternative lysis methods

a) Sample contains organisms that are difficult to lyse

A 10 minute incubation at 70°C after adding Solution C1 can be performed. Continue by proceeding with the mechanical lysis step using the 96 well Plate Shaker.

b) Freeze-thaw lysis

To improve the lysing efficiency of this kit, freeze/thaw cycles may be performed after adding samples to the bead plate and adding the Bead Solution. Store the plate at –70°C or –20°C until the samples are completely frozen. Immediately float the DNeasy PowerSoil HTP Bead Plate in a 65°C water bath to thaw. Repeat the freeze-thaw a second time and proceed with the addition of Solution C1.

Optional: After the second freeze-thaw, Solution C1 can be added along with Proteinase-K (cat. no. 19131) to improve the lysis efficiency for some organisms.

Appendix A: Soil Amounts

This DNeasy PowerSoil HTP 96 Kit is designed to process 0.25 g of soil. Recommended starting amounts for different soil types are listed in Table 1. For wet soils, see information in the Troubleshooting Guide.

Table 1. Recommended amount of soil by type

Type of soil sample	Recommended maximum to process (g)
Dry sandy	0.5
Dry clay	0.25
Wet clay	0.25
Potting	0.1
Sediment	0.25
Loam	0.25
Peat moss	0.1
Rich farm	0.25
Amended	0.15
Compost	0.1

For larger sample sizes (up to 5 g) try using the DNeasy PowerMax® Soil Kit (cat. no. 12988-10).

Appendix B: Additional Applications and References

Root tips/mycorrhizae

Microbial colonization of root tips and microbial growth penetrating the root tips are both common phenomenon occurring in many plant species. For optimal processing of these samples, we recommend the DNeasy Plant Pro Kits, the DNeasy 96 Plant Kit, or the RNeasy® PowerPlant® Kit (cat. nos. 47014, 47016, 69181, or 13500-50, respectively).

Reference

1. De Souza, F.A., Kowalchuk, G.A., Leeflang, P., van Veen, J.A. and Smit, E. (2004) PCR-denaturing gradient gel electrophoresis profiling of inter- and intra-species 18s rRNA gene sequence heterogeneity is an accurate and sensitive method to assess species diversity of arbuscular mycorrhizal fungi of the genus Gigaspora. Appl. Environ. Microbiol. **70**, 1413–1424.

Microbial mats and biofilms

Microbial mats and biofilms are a common occurrence in many natural environments and provide protection for the microbial communities growing within them. Direct lysis of the microbial cells within this protected community is inefficient. For processing biofilm and biomat samples, we recommend the DNeasy and RNeasy PowerBioFilm® Kits (cat. nos. 24000-50 or 25000-50, respectively).

Fungal mats

The DNeasy PowerSoil HTP 96 Kit is suitable for isolating DNA from fungi grown in pure culture. Harvesting a freshly grown culture is suggested because the growing hyphal tips of the fungal mat are a rich source of DNA. This is accomplished by harvesting the fungal mat using cheesecloth, which will separate single cell organisms from the fungal mat. After filtering, transfer an appropriate amount of the retained moist fungal mat into the Bead Plate. To improve the lysing efficiency of this kit, freeze/thaw cycles may be performed after adding samples to the bead plate and adding the Bead Solution. Store the plate at –70°C or –20°C until the samples are completely frozen. Immediately float the DNeasy PowerSoil HTP Bead Plate in a 65°C water bath to thaw. Repeat the freeze-thaw a second time and proceed with the addition of Solution C1.

Spores (bacterial and fungal) in soils

Fungal spores are tougher than bacterial spores because of their high polysaccharide content. Follow the freeze-thaw protocol detailed above. Heating the samples to 70°C for 15 minutes before bead beating may also be helpful. When dealing with spores harvested from pure cultures, please use the DNeasy UltraClean® Microbial Kit (cat. no. 12224-50).

Fecal samples and cattle manure

The DNeasy PowerSoil HTP 96 Kit can be used without significant modifications on a wide variety of fecal materials.

References

1. Clement, B.G. and Kitts, C.L. (2000) Isolating PCR quality DNA from human feces with a soil DNA kit. Biotechniques **28**, 640–644.

2. Ibekwe, A.M., Watt, P., Grieve, C.M., Sharma, V. and Lyons, S.R. (2002) Multiplex fluoregenic real-time PCR for detection and quantification of Escherichia coli O157:H7 in dairy wastewater wetlands. Appl. Environ. Microbiol. **68**, 4853–4862.

3. Ibekwe, A.M., Grieve, C.M. and Lyons, S.R. (2003) Characterization of microbial communities and composition in constructed dairy wetland wastewater effluent. Appl. Environ. Microbiol. **69**, 5060–5069.

4. Trochimchuk, T., Fotheringham, J., Topp, E., Schraft, H. and Leung, K.T. (2003) A comparison of DNA extraction and purification methods to detect Escherichia coli O157:H7 in cattle manure. J. Microbiol. Methods **54**, 165–175.

Rumen samples

The DNeasy PowerSoil HTP 96 Kit can be used to extract PCR-quality DNA from rumen samples as described in the reference below. Rumen has an actively metabolizing microbial community; it is also rich in phenolic compounds and polysaccharides. These contaminants often inhibit downstream molecular analyses. Using the DNeasy PowerSoil Kit, with the modifications in the publication below, will result in application ready DNA.

Reference

1. Mackie, R.I. et al. (2003) Ecology of uncultivated Oscillospira species in the rumen of cattle, sheep and reindeer as assessed by microscopy and molecular approaches. Appl. Environ. Microbiol. **69**, 6808–6815.

Ordering Information

Product	Contents	Cat. no.
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
Related Products		
DNeasy PowerSoil Pro (50)	For 50 preps: Isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro (250)	For 100 preps: Isolation of microbial genomic DNA from all soil types	47016
DNeasy PowerMax Soil Kit (10)	For 10 preps: Isolation of 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: Isolation of high-quality total RNA from all soil types	12866-25
RNeasy PowerBiofilm Kit (50)	For 50 preps: Isolation of high-quality total RNA from biofilm samples	25000-50
DNeasy PowerBiofilm Kit (50)	For 50 preps: Isolation of high-quality, pure DNA from biofilm samples	24000-50
DNeasy Plant Pro Kit (50)	For 50 preps: Isolation of genomic DNA from plant and seed samples, removes polyphenolics and polysaccharides	69204
DNeasy Plant Pro Kit (250)	For 250 preps: Isolation of genomic DNA from plant and seed samples, removes polyphenolics and polysaccharides	69206

Product	Contents	Cat. no.
DNeasy 96 Plant Kit (6)	6 DNeasy 96 Plates, buffers, reagents, RNase A, S-Blocks, collection microtubes (1.2 ml), caps, AirPore tape sheets	69181
RNeasy PowerPlant Kit (50)	For 50 preps: Isolation of total RNA from plant and seed samples, including those high in polyphenols and polysaccharides	13500-50
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300
Plate Adapter Set	Set of four adapters required to assemble two 96 well plates onto the 96 well Plate Shaker	11990

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Handbook Revision History

Date	Changes
05/2017	Initial release
04/2018	Kit Contents quantities updated: • Collection Plates (2 ml) (4 instead of 2 x 4) DNA storage temperatures updated to reflect appropriate temperature ranges. Editorial changes.
08/2019	Correction of suggestion provided for freeze–thaw lysis in the Troubleshooting Guide.