July 2021

DNeasy® 96 PowerSoil® Pro Kit Handbook

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

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy 96 PowerSoil Pro Kit	**(384)**
Catalog no.	**47017**
Number of preps	**384**
PowerBead Pro Plates	4
Sealing Film	4
Silicone Compression Mats	2
QIAamp® 96 Spin Plates	4
Solution CD1	2 x 200 ml
Solution CD2	2 x 60 ml
Solution CD3	2 x 175 ml
Solution EA	1 x 175 ml; 1 x 36 ml
Solution C5	1 x 175 ml; 1 x 30 ml
Solution C6	1 x 66 ml
Collection microtubes, racked (CMTRs)	8
Caps for collection microtubes (55 x 8)	2
S-Blocks	8
Sealing Tape	32
Quick-Start Protocol	1
Quick-Start Guide PowerBead Pro Plates	1

Storage

Solution CD2 should be stored at 2–8°C upon arrival. All other reagents and kit components of the DNeasy 96 PowerSoil Pro Kit can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All DNeasy products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of DNeasy 96 PowerSoil Pro Kits is tested against predetermined specifications to ensure consistent product quality.

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.

CAUTION 	Solution EA and Solution C5 are flammable.

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

Solution CD1 and Solution CD3 contain chaotropic 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.

Introduction

The DNeasy 96 PowerSoil Pro Kit allows manual 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 uses centrifugation exclusively. The second protocol combines both centrifugation and vacuum steps. This kit requires the use of a specialized plate shaker to facilitate the bead-beating process in the PowerBead Pro Plates. We recommend the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990).

The DNeasy 96 PowerSoil Pro Kit comprises a novel and proprietary method for isolating microbial genomic DNA from environmental samples. The kit uses QIAGEN's second-generation Inhibitor Removal Technology® (IRT) and is intended for use with environmental samples containing high humic acid content, including difficult soil types such as compost, sediment, and manure. Other more common soil and stool types have also been used successfully with this kit. Improved IRT combined with more efficient bead beating and lysis chemistry yields high-quality DNA that can be used immediately in downstream applications, including PCR, qPCR, and next-generation sequencing (NGS; 16S and whole-genome).

Principle and procedure

The DNeasy 96 PowerSoil Pro Kit is effective at removing PCR inhibitors from soil and stool materials, from even the most difficult types. Environmental or human samples are added to a 96-well bead-beating plate 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-plate format. DNA is then washed and eluted from the membrane and ready for NGS, PCR, and other downstream applications.

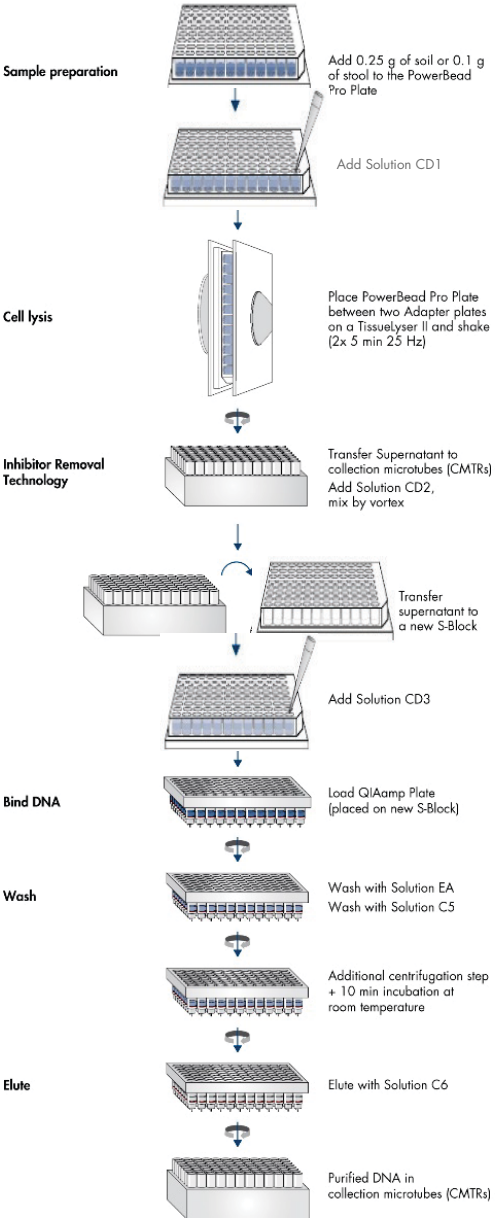

Figure 1. DNeasy 96 PowerSoil Pro Kit Procedure.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- 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.
- TissueLyser II (cat. no. 85300) and Plate Adapter Sets (cat. no. 11990)
- Multi-channel pipettor (50–1000 µl)
- Extra-long pipette tips (0.1–1000 µl) for CMTRs (optional)
- Vortex-Genie® 2 vortex with 3 inch platform (optional)
- 70% ethanol (optional)
- Reagent reservoirs (optional)
- Vacuum pump (optional)
- Vacuum manifold (optional)

Protocol: Experienced User, Centrifugation

Important notes before starting

- If Solution CD3 has precipitated, heat at 60°C until the precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).
- 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 or CMTR/QIAamp 96 Plate does not fit into your centrifuge buckets.

Procedure

1. Centrifuge a PowerBead Pro Plate briefly to ensure that the beads have settled at the bottom.
2. Remove and discard the square well mat from the PowerBead Pro Plate. Add up to 0.25 g of soil sample or up to 0.1 g of stool sample in each well.

   Note: Avoid cross contamination between sample wells.
3. Add 800 µl of Solution CD1 to the wells of the PowerBead Pro Plate.
4. Remove any residual liquid on top of the plate and secure the sealing film (provided) tightly onto the bead plate. Use a tool such as a scraper or plate roller to make the sealing film adhere firmly onto the plate.

   Note: Liquid on top of the plate will prevent a tight sealing of the plate with the sealing film. A strong seal is essential to prevent leakage during disruption in the TissueLyser II. Usage of a mechanical plate sealer can be advantageous for a consistent and a uniform seal.
5. Put the silicone compression mat (provided) on top of the bead plate that is sealed with the sealing film.

   Note: Two silicone compression mats are provided so that 2 plates can be processed in parallel in the TissueLyser II. The mats are reused for the remaining plates.

6. Place this entire assembly (from steps 1 to 5) between 2 Plate Adapter Sets for disruption in the TissueLyser II.

   Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because extended processing might lead to leakage.

7. Shake at a speed of 25 Hz for 5 min. Re-orient the plates so that the side that was closest to the machine body is now furthest from it and shake again at speed of 25 Hz for 5 min.
8. Centrifuge at room temperature for 6 min at 4500 x *g*.
9. Discard the sealing film. Transfer the supernatant to the collection microtubes.

   Note: Expect 500–600 µl. The supernatant may still contain soil or stool particles.

10. Add 200 µl of Solution CD2. Seal collection microtubes with the caps provided, and vortex.
11. Centrifuge the plate at room temperature for 6 min at 4500 x *g*.
12. Transfer up to 700 µl of supernatant to an S-Block.

    Note: Expect 500–600 µl.

13. Add 600 µl of Solution CD3 to each well of the plate. Pipet samples up and down to mix. Place a QIAamp 96 Spin Plate onto a new S-Block.
14. Load approximately 650 µl into each well of the QIAamp 96 Spin Plate and seal the plate with a sealing tape.
15. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the QIAamp 96 Spin Plate back on the same S-Block. Discard the sealing tape.
16. Repeat steps 14 and 15 until all the supernatant has been processed. Discard the final flow-through.
17. Place the QIAamp 96 Spin Plate back on the same S-Block.
18. Add 500 µl of Solution EA to each well of the QIAamp 96 Spin Plate and seal the plate with a sealing tape.
19. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through, and place the QIAamp 96 Spin Plate back on the same S-Block.

20. Add 500 µl of Solution C5 to the QIAamp 96 Spin Plate and seal the plate with a sealing tape. Centrifuge for 3 min at 4500 x *g*.
21. Discard the flow-through (remove any trace of the flow-through from the S-Block) and place the QIAamp 96 Spin Plate (still sealed from step 20) into the same S-Block.
22. Centrifuge again at room temperature for 5 min at 4500 x *g*. Discard the flow-through.
23. Carefully place the QIAamp 96 Spin Plate onto collection microtubes. Discard the sealing tape.
24. Allow to air dry for 10 min at room temperature.
25. Add 100 µl of Solution C6 to the center of each well. Seal the plate with a sealing tape.
26. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the sealing tape.
27. Seal collection microtubes with the caps provided. The DNA is now ready for downstream applications.

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

Protocol: Detailed, Centrifugation

Important notes before starting

- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).
- Wear gloves at all times.
- The assembled S-Block and QIAamp 96 Spin Plate may not fit some centrifuge types. Please contact QIAGEN Technical Support at **support.qiagen.com** if the S-Block/QIAamp 96 Spin Plate or CMTR/QIAamp 96 Plate does not fit into your centrifuge buckets.

Procedure

1. Centrifuge a PowerBead Pro Plate briefly to ensure that the beads have settled at the bottom.
2. Remove and discard the square well mat from the PowerBead Pro Plate. Add up to 0.25 g of soil sample or up to 0.1 g of stool sample in each well.

   Note: Avoid cross contamination between sample wells.
3. Add 800 µl of Solution CD1 to the wells of the PowerBead Pro Plate.
4. Remove any residual liquid on top of the plate and secure the sealing film (provided) tightly onto the bead plate. Use a tool such as a scraper or plate roller to make the sealing film adhere firmly onto the plate.

   Note: Liquid on top of the plate will prevent a tight sealing of the plate with the sealing film. A strong seal is essential to prevent leakage during disruption in the TissueLyser II. Usage of a mechanical plate sealer can be advantageous for a consistent and a uniform seal.
5. Put the silicone compression mat (provided) on top of the bead plate that is sealed with the sealing film.

   Note: Two silicone compression mats are provided so that 2 plates can be processed in parallel in the TissueLyser II. The mats are reusable for the remaining plates.

6. Place this entire assembly (from steps 1 to 5) between 2 Plate Adapter Sets (cat. no. 11990) for disruption in the TissueLyser II.

   Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because extended processing might lead to leakage.

7. Shake at a speed of 25 Hz for 5 min. Re-orient the plates so that the side that was closest to the machine body is now furthest from it and shake again at a speed of 25 Hz for 5 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.

8. 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. The supernatant contains the DNA.

9. Discard the sealing film. Transfer the supernatant to the collection microtubes.

   Note: Expect 500–600 µl. The supernatant may still contain some soil or stool particles.

10. Add 200 µl of Solution CD2. Seal collection microtubes with the caps provided and vortex.

    Note: Solution CD2 contains IRT and can precipitate non-DNA organic and inorganic materials 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.

    Note: For samples with a very high inhibitor content, we recommend using 250 µl Solution CD2.

11. Centrifuge the collection microtubes at room temperature for 6 min at 4500 x *g*.

12. Transfer up to 700 µl of supernatant to an S-Block.

    Note: Expect 500–600 µl. For easy handling leave the 8-well microtube stripe in the rack (the rack is see-through, and the pellet should be visible). After removing the supernatant of the first microtube strip, remove the used strip, then move the next 8-well strips sequentially to the front and transfer the supernatant.

    Note: The pellet at this point contains non-DNA organic and inorganic material including humic acids, cell debris, and proteins. For best DNA yields and quality, avoid transferring any of the pellet.

13. Add 600 µl of Solution CD3 to each well of the plate. Pipet samples up and down to mix. Place a QIAamp 96 Spin Plate onto a new S-Block.

    Note: Solution CD3 is a highly concentrated salt solution. Since DNA binds tightly to silica at high salt concentrations, Solution CD3 will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the QIAamp 96 Spin Plate filter membrane.

14. Load approximately 650 µl into each well of the QIAamp 96 Spin Plate and seal the plate with a sealing tape.

15. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the QIAamp 96 Spin Plate back on the same S-Block. Discard the sealing tape.

16. Repeat steps 14 and 15 until all the supernatant has been processed. Discard the final flow-through.

    Note: In the highly concentrated salt solution, DNA is selectively bound to the silica membrane in the QIAamp 96 Spin Plate. Contaminants pass through the silica membrane, leaving only DNA bound to the membrane.

17. Place the QIAamp 96 Spin Plate back on the same S-Block.

18. Add 500 µl of Solution EA to each well of the QIAamp 96 Spin Plate and seal the plate with a sealing tape.

    Note: Solution EA is a wash buffer that removes protein and other non-aqueous contaminants from the QIAamp 96 Spin Plate filter membrane.

19. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the QIAamp 96 Spin Plate back on the same S-Block.

20. Add 500 µl of Solution C5 to the QIAamp 96 Spin Plate and seal the plate with a sealing tape. Centrifuge for 3 min at 4500 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 QIAamp 96 Spin Plate. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

21. Discard the flow-through (remove any remnant of flow-through from S Block) and place the QIAamp 96 Spin Plate (still sealed from step 20) into the same S Block.

    Note: To remove remnants of the flow-through, place a clean paper towel on top of the S-Block or centrifuge the S-Block before placing the QIAamp 96 Spin Plate back into the S-Block.

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

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

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

23. Carefully place the QIAamp 96 Spin Plate onto new collection microtubes. Discard the sealing tape.

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

    Note: This step removes residual Solution C5.

25. Add 100 µl of Solution C6 to the center of each well. Seal plate with a sealing tape.

    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 QIAamp 96 Spin Plate membrane.

26. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the sealing tape.

    Note: As Solution C6, which is a low salt solution, passes through the silica membrane, the DNA that was bound in the presence of high salt is now selectively released.

27. Seal collection microtubes with the caps provided. The DNA is now ready for downstream applications.

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

Protocol: Experienced User, Centrifugation, and Vacuum

Important notes before starting

- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all 4 support at **support.qiagen.com** if the S-Block/QIAamp 96 Plate or CMTR/QIAamp 96 Plate does not fit into your centrifuge buckets.
- Wear gloves at all times.

Procedure

1. Centrifuge a PowerBead Pro Plate briefly to ensure that the beads have settled at the bottom.
2. Remove and discard the square well mat from the PowerBead Pro Plate. Add up to 0.25 g of soil sample or up to 0.1 g of stool sample in each well.

   Note: Avoid cross contamination between sample wells.
3. Add 800 µl of Solution CD1 to the wells of the PowerBead Pro Plate.
4. Remove any residual liquid on top of the plate and secure the sealing film (provided) tightly onto the bead plate. Use a tool such as a scraper or plate roller to make the sealing film adhere firmly onto the plate.

   Note: Liquid on top of the plate will prevent a tight sealing of the plate with the sealing film. A strong seal is essential to prevent leakage during disruption in the TissueLyser II. Usage of a mechanical plate sealer can be advantageous for a consistent and a uniform seal.
5. Put the silicone compression mat (provided) on top of the bead plate that is sealed with the sealing film.

   Note: Two silicone compression mats are provided so that 2 plates can be processed in parallel in the TissueLyser II. The mats are reusable for the remaining plates.

6. Place this entire assembly (from steps 1 to 5) between 2 Plate Adapter Sets for disruption in the TissueLyser II.

   Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because extended processing might lead to leakage.

7. Shake at a speed of 25 Hz for 5 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at a speed of 25 Hz for 5 min.
8. Centrifuge at room temperature for 6 min at 4500 x *g*.
9. Discard the sealing film. Transfer the supernatant to the collection microtubes.

   Note: Expect 500–600 µl. The supernatant may still contain soil or stool particles.

10. Add 200 µl of Solution CD2. Seal collection microtubes with the caps provided and vortex.
11. Centrifuge the plate at room temperature for 6 min at 4500 x *g*.
12. Transfer up to 700 µl of supernatant to an S-Block.

    Note: Expect 500–600 µl.

13. Add 600 µl of Solution CD3 to each well of the plate. Pipet samples up and down to mix. Place a QIAamp 96 Spin Plate onto a new S-Block.
14. Load approximately 650 µl into each well of the QIAamp 96 Spin Plate and seal the plate with a sealing tape.
15. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the QIAamp 96 Spin Plate back on the same S-Block. Discard the sealing tape.
16. Repeat steps 14 and 15 until all the supernatant has been processed. Discard the final flow-through and remove any remnant of flow-through from the S-Block.
17. Remove the top portion of a vacuum manifold and place the S-Block used in steps 15 and 16 to the bottom of the vacuum manifold.
18. Replace the top of the manifold and place a QIAamp 96 Spin Plate on it. Turn the vacuum pump on.

Note: Test that you have a good seal with the manifold and the QIAamp 96 Spin Plate. You should be able to gently lift the entire unit without the QIAamp 96 Spin Plate separating from the manifold.

19. Add 500 µl of Solution EA to each well of the QIAamp 96 Spin Plate. Once the entire volume of Solution EA has passed through the well, turn off the vacuum.
20. Add 500 µl of Solution C5 to each well of the QIAamp 96 Spin Plate. Turn the vacuum back on. Once the entire volume of Solution C5 has passed through the well, turn off the vacuum.
21. Remove the QIAamp 96 Spin Plate and set aside. Discard the flow-through from the S-Block and place it back into the manifold.
22. Replace the QIAamp 96 Spin Plate on top of the manifold. Turn the vacuum on and test the seal.
23. Apply maximum vacuum for an additional 10 min to dry the membrane.
24. Switch off vacuum and ventilate the manifold slowly. Lift the top plate from the bottom of the vacuum manifold (not the QIAamp 96 Spin Plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the QIAamp 96 Spin Plate with clean absorbent paper.

    Note: This step removes any trace of wash buffer, which may be present around the outlet nozzles and collars of the QIAamp 96 Spin Plate.
25. Replace the S-Block with collection microtubes. Place the top plate (with the QIAamp 96 Spin Plate on it) back to the bottom of the vacuum manifold, making sure that the QIAamp 96 Spin Plate is positioned securely.
26. To elute, add 100 µl of Solution C6 to the center of each well, let stand for 1 min, and apply maximum vacuum for 5 min. Switch off the vacuum and ventilate the manifold slowly.
27. Seal collection microtubes with the caps provided. The DNA is now ready for downstream applications.

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

Protocol: Detailed Centrifugation and Vacuum

Important notes before starting

- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).
- Wear gloves at all times.
- The assembled S-Block and QIAamp 96 Spin Plate may not fit some centrifuge types. Please contact QIAGEN Technical Support at **support.qiagen.com** if the S-Block/QIAamp 96 Spin Plate or CMTR/QIAamp 96 Plate does not fit into your centrifuge buckets.

Procedure

1. Centrifuge a PowerBead Pro Plate briefly to ensure that the beads have settled at the bottom.
2. Remove and discard the square well mat from the PowerBead Pro Plate. Add up to 0.25 g of soil sample or up to 0.1 g of stool sample in each well.

   Note: Avoid cross contamination between sample wells.
3. Add 800 µl of Solution CD1 to the wells of the PowerBead Pro Plate.
4. Remove any residual liquid on top of the plate and secure the sealing film (provided) tightly onto the bead plate. Use a tool such as a scraper or plate roller to make the sealing film adhere firmly onto the plate.

   Note: Liquid on top of the plate will prevent a tight sealing of the plate with the sealing film. A strong seal is essential to prevent leakage during disruption in the TissueLyser II. Usage of a mechanical plate sealer can be advantageous for a consistent and a uniform seal.
5. Put the silicone compression mat (provided) on top of the bead plate that is sealed with the sealing film.

   Note: Two silicone compression mats are provided so that 2 plates can be processed in parallel in the TissueLyser II. The mats are reusable for the remaining plates.

6. Place this entire assembly (from steps 1 to 5) between 2 Plate Adapter Sets (cat. no. 11990) for disruption in the TissueLyser II.

   Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because doing so might lead to leakage.

7. Shake at speed of 25 Hz for 5 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 25 of Hz for 5 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.

8. 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.

9. Discard the sealing film. Transfer the supernatant to the collection microtubes.

   Note: Expect 500–600 µl. The supernatant may still contain some soil or stool particles.

10. Add 200 µl of Solution CD2. Seal collection microtubes with the caps provided and vortex.

    Note: Solution CD2 contains IRT, and 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.

    Note: For samples with a very high inhibitor content, we recommend using 250 µl Solution CD2.

11. Centrifuge the collection microtubes at room temperature for 6 min at 4500 x *g*.

12. Transfer up to 700 µl of supernatant to an S-Block.

    Note: Expect 500–600 µl. For easy handling leave the 8-well microtube stripe in the rack (the rack is see-through, and the pellet should be visible). After removing the supernatant of the first microtube strip, remove the used strip, then move the next 8-well strips sequentially to the front and transfer the supernatant.

Note: The pellet at this point contains non-DNA organic and inorganic material, including humic acids, cell debris, and proteins. For best DNA yields and quality, avoid transferring any of the pellet.

13. Add 600 µl of Solution CD3 to each well of the plate. Pipet samples up and down to mix. Place a QIAamp 96 Spin Plate onto a new S-Block.

    Note: Solution CD3 is a highly concentrated salt solution. Because DNA binds tightly to silica at high salt concentrations, Solution CD3 will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the QIAamp 96 Spin Plate filter membrane.

14. Load approximately 650 µl into each well of the QIAamp 96 Spin Plate and seal the plate with a sealing tape.

15. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the QIAamp 96 Spin Plate back on the same S-Block. Discard the sealing tape.

16. Repeat steps 14 and 15 until all the supernatant has been processed. Discard the final flow-through and remove any remnant of flow-through from the S-Block.

    Note: In the high-salt solution, DNA is selectively bound to the silica membrane in the QIAamp 96 Spin Plate. Contaminants pass through the silica membrane, leaving only DNA bound to the membrane.

    Note: To remove remnants of flow-through, place a clean paper towel on top of S-Block or centrifuge empty S-Block.

17. Remove the top portion of a vacuum manifold and place the S-Block used in steps 15 and 16 in the bottom of the vacuum manifold.

    Note: A waste tray instead of the S-Block can also be used if available.

18. Replace the top of the manifold and place a QIAamp 96 Spin Plate on it. Turn the vacuum pump on.

    Note: Test that you have a good seal with the manifold and the QIAamp 96 Spin Plate. You should be able to gently lift the entire unit without the QIAamp 96 Spin Plate separating from the manifold.

19. Add 500 µl of Solution EA to each well of the QIAamp 96 Spin Plate. Once the entire volume of Solution EA has passed through the well, turn off the vacuum.

    Note: Solution EA is a wash buffer that removes protein and other non-aqueous contaminants from the QIAamp 96 Spin Plate filter membrane.

20. Add 500 µl of Solution C5 to each well of the QIAamp 96 Spin Plate. Turn the vacuum pump back on. Once the entire volume of Solution C5 has passed through the well, turn off the vacuum.

    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 QIAamp 96 Spin Plate. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

    Optional: Depending on the sample, an additional 70% ethanol wash may increase DNA purity. Add 500 µl to each well of the QIAamp 96 Spin Plate. Turn on the vacuum. Once the entire volume of the 70% ethanol has passed through the well, turn off the vacuum.

    Note: When performing a third wash step, the flow-through from steps 19 and 20 should be discarded from the S-Block beforehand.

21. Remove the QIAamp 96 Spin Plate and set aside. Discard the flow-through from the S-Block, remove any remnant of flow-through from the S-Block, and place it back into the manifold.

22. Replace the QIAamp 96 Spin Plate on top of the manifold. Turn on the vacuum and test the seal.

23. Apply maximum vacuum for an additional 10 min to dry the membrane.

    Note: This step removes residual wash buffer from the membrane. It is critical to remove all traces of wash solution because it can interfere with downstream DNA applications. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator or leakage valves if they are used), allowing maximum airflow to go through the wells.

24. Switch off the vacuum and ventilate the manifold slowly. Lift the top plate from the bottom of the vacuum manifold (not the QIAamp 96 Spin Plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the QIAamp 96 Spin Plate with clean absorbent paper.

    Note: This step removes any trace of wash buffer, which may be present around the outlet nozzles and collars of the QIAamp 96 Spin Plate.

    Note: It is important to avoid any traces of the Solution C5.

25. Replace the S-Block (if the waste tray was used instead of an S-Block, replace the waste tray with collection microtubes) with collection microtubes. Place the top plate (with the QIAamp 96 Spin Plate on it) back on the bottom of the vacuum manifold, making sure that the QIAamp 96 Spin Plate is positioned securely.

    Note: To avoid cross contamination, it is important that the outlet nozzles of the QIAamp 96 Spin Plate line up with the wells of the collection microtubes.

26. To elute, add 100 µl of Solution C6 to the center of each well, let stand for 1 min, and apply maximum vacuum for 5 min. Switch off vacuum and ventilate the manifold slowly.

    Note: Placing Solution C6 (elution buffer) at the center of the 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 QIAamp 96 Spin Plate membrane.

27. Seal collection microtubes with the caps provided. The DNA is now ready for downstream applications.

    Note: We recommend storing DNA frozen (–30 to –15°C or –90 to –65°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)	Amount of soil and stool to process	The QIAGEN DNeasy 96 PowerSoil Pro Kit is designed to process 0.25 g of soil and up to 0.1 g of stool. For inquiries regarding the use of larger sample amounts, please contact QIAGEN Technical Support for suggestions.
b)	Soil or stool sample is high in water content	Weigh the slurry and dispense into the wells. We suggest restricting the starting amount to 0.25 g soil and 0.1 g stool. Increasing the amount used will increase the amount of volume in the subsequent steps.
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.
d)	A vacuum step seems to be taking a long time	Turn off the vacuum source and lift the QIAamp 96 Spin Plate off the vacuum to release any back pressure. Replace the QIAamp 96 Spin Plate and turn the vacuum back on. Make sure there are no air leaks around the plate. If slow vacuum continues, you can centrifuge the QIAamp 96 Spin Plate as an alternative. Make sure any unused wells are covered with sealing tape.

		Comments and suggestions
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 DNeasy 96 PowerSoil Pro Kit. However, it should still be attempted. If DNA will still not amplify after trying the steps above, then PCR optimization may be needed.
b)	Eluted DNA is brown	If you observe coloration in your samples, please contact QIAGEN Technical Support for suggestions.
c)	Concentrating eluted DNA	The final volume of eluted DNA will be 50–100 µl. The DNA may be concentrated by adding 5–10 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 100 µl of 100% cold ethanol and invert 3–5 times to mix. Incubate at –30 to –15°C for 30 min and centrifuge at 10,000 x *g* for 5 min at room temperature. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris (Solution C6).
d)	DNA floats out of a well when loading a gel	This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 15 and not transferring liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in "Concentrating eluted DNA") is the best way to remove residual Solution C5.
e)	Storing DNA	DNA is eluted in Solution C6 (10 mM Tris) and must be stored at –30 to –15°C or –90 to –65°C to prevent degradation. DNA can be eluted in Buffer TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted in sterile, DNA-Free PCR-grade water (cat. no. 17000-10).
Alternative lysis methods		
a)	Cells are difficult to lyse	After adding Solution CD1 and prior to the bead-beating step, incubate at 65°C for 10 min. Resume protocol from step 2.
b)	Reduction of shearing of DNA	After adding Solution CD1, vortex 3–4 s, then heat to 70°C for 5 min. Repeat once. This alternative procedure will reduce shearing but may also reduce yield.

Ordering Information

Product	Contents	Cat. no.
DNeasy 96 PowerSoil Pro Kit	For 4 x 96 preps: Manual high-throughput isolation of DNA from soil samples in less than one day	47017
Related products		
DNeasy PowerSoil Pro Kit (50)	For 50 preps: Isolate microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For 250 preps: Isolate microbial genomic DNA from all soil types	47016
QIAamp PowerFecal Pro DNA Kit (50)	For 50 preps: Isolation of microbial genomic DNA from stool and gut samples	51804
DNeasy 96 PowerSoil Pro QIAcube® HT Kit (480)	For 480 preps: Automated high-throughput purification of microbial genomic DNA from all soil and stool types	47021
MagAttract® PowerSoil Pro DNA Kit	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	47109
MagAttract PowerSoil DNA EP Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27100-4-EP
DNeasy PowerMax® Soil Kit (10)	For 10 preps: Isolation of microbial DNA from large quantities of stool; great for samples with low microbial load	12988-10
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300

Product	Contents	Cat. no.
Plate Adapter Set	Set of four adapters required to assemble two 96 well plates onto the 96 well Plate Shaker.	11990
UCP Multiplex PCR Kit (100)	For 100 reactions: For highly specific and sensitive multiplex PCR with minimized background using nucleic acid-depleted reagents	206742
UCP Multiplex PCR Kit (500)	For 500 reactions: For highly specific and sensitive multiplex PCR with minimized background using nucleic acid-depleted reagents	206744
QIAseq 16S/ITS Screening Panel (24)	Profiling of bacterial and fungal communities by constructing a library of all bacterial 16S rRNA gene variable regions and fungal ITS regions using phased primer. Sufficient for 24 samples	333812
QIAseq 16S/ITS Screening Panel (96)	Profiling of bacterial and fungal communities by constructing a library of all bacterial 16S rRNA gene variable regions and fungal ITS regions using phased primer. Sufficient for 96 samples	333815
QIAseq 16S/ITS Region Panel (24)	Profiling of bacterial and fungal communities by constructing a library of specific bacterial 16S rRNA gene variable regions and fungal ITS regions using phased primer. Sufficient for 24 samples	333842
QIAseq 16S/ITS Region Panel (96)	Profiling of bacterial and fungal communities by constructing a library of specific bacterial 16S rRNA gene variable regions and fungal ITS regions using phased primer. Sufficient for 96 samples	333845

Product	Contents	Cat. no.
QIAseq 16S/ITS 24-Index I (96)	Adapters and sample indexes for use in conjunction with QIAseq 16S/ITS Panels to generate Illumina-compatible libraries; sufficient adapters for indexing 96 samples (4 x 24 samples)	333822
QIAseq 16S/ITS 96-Index I (384)	Adapters and sample indexes (Set A) for use in conjunction with QIAseq 16S/ITS Panels to generate Illumina-compatible libraries; sufficient adapters for indexing 384 samples (4 x 96 samples)	333825
QIAseq FX DNA Library UDI Kit (96)	Buffers and reagents for DNA fragmentation (including end repair and A-addition), ligation and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal, allowing usage of defined parts of plate)	Inquiry
QIAseq FX DNA Library CDI Kit (96)	Buffers and reagents for DNA fragmentation (including end repair and A-addition), ligation and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal, allowing usage of defined parts of plate)	180484

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.

Handbook Revision History

Date	Changes
09/2020	Initial release.
07/2021	Updated the text under Storage section. Used “QIAamp 96 Spin Plate” throughout the handbook. Added a statement about using a mechanical plate sealer on some procedure steps. Corrected the Ordering Information entry for QIAseq FX DNA Library UDI Kit and QIAseq FX DNA Library CDI Kit. Updated the Ordering Information entry for MagAttract PowerSoil Pro DNA Kit.

Notes

Limited License Agreement for DNeasy 96 PowerSoil Pro Kit

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