
August 2019

DNeasy[®] 96 PowerSoil[®] Pro QIAcube[®] HT Kit Handbook

For automated purification of
microbial genomic DNA from all
soil types and stool samples using
QIAcube HT with QIAcube HT Prep
Manager Software

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Kit Contents

DNeasy 96 PowerSoil Pro QIAcube HT Kit	(480)
Catalog no.	47021
Number of preps	480
QIAamp 96 Plates	5
Solution CD1*	2 x 200 ml
Solution CD2	3 x 60 ml
Solution CD3*	2 x 175 ml
Buffer AW1*† (concentrate)	242 ml
Buffer AW2† (concentrate)	127 ml
Solution C6	66 ml
TopElute Fluid	60 ml
S-Blocks	5
Sealing Tape	16
Quick-Start Protocol	1

* **Caution:** Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 5 for safety information.

† Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.

QIAcube HT Plasticware	(480)
Catalog no.	950067
Number of preps	480
S-Blocks	5
Filter-Tips OnCor C	9 x 96
Tape Pad	1
Elution Microtubes RS (EMTR)	5
8-Well Strip Caps for EMTR	120

For mechanical disruption*	Cat. no.
PowerBead Pro Tubes	19301
PowerBead Pro Plates	19311

* For mechanical disruption, PowerBead Pro Plates or PowerBead Pro Tubes must be ordered separately.

Note: The following components can also be ordered separately: S-Blocks, Elution Microtubes RS (including caps for strips) and TopElute Fluid. Please see "Ordering Information".

Storage

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

Intended Use

The DNeasy 96 PowerSoil Pro QIAcube HT Kit is intended for the automated extraction of microbial genomic DNA using the QIAcube HT instrument with the Prep Manager Software.

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.

<p>WARNING</p> 	<p>Buffer AW1, Buffer AW2, and ethanol are flammable.</p>
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<p>CAUTION</p> 	<p>DO NOT add bleach or acidic solutions directly to the sample preparation waste.</p>
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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.

Quality Control

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

Introduction

The DNeasy 96 PowerSoil Pro QIAcube HT Kit comprises a novel and proprietary method for the automated, high-throughput isolation of microbial genomic DNA from stool and environmental samples. The kit uses QIAGEN's second-generation Inhibitor Removal Technology® (IRT) and is intended for use with environmental samples with 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 in an automated 96-well format, yields high-quality DNA that can be used immediately in downstream applications, including PCR, qPCR, and next-generation sequencing (NGS) applications (16S and whole genome).

Principle and procedure

The DNeasy 96 PowerSoil Pro QIAcube HT Kit is effective at removing PCR inhibitors from even the most difficult sample types. It does so in an automated 96-well format. Samples are added to a PowerBead Pro Plate or a PowerBead Pro Tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on silica membranes in a 96-well plate spin column format on the QIAcube HT instrument using vacuum technology. DNA is then automatically washed and eluted from the membranes and is ready for NGS, PCR, and other downstream applications.

Starting material

This protocol is intended primarily for the extraction of microbial genomic DNA from environmental samples such as soil material. We recommend starting with a maximum of 250 mg of sample types that can be processed using this protocol.

Typically, stool samples also contain high amounts of inhibitors that interfere with downstream enzymatic reactions and compounds that can degrade DNA. The DNeasy 96 PowerSoil Pro QIAcube HT protocol is suitable for the removal of these substances and the extraction of microbial genomic DNA that is free of proteins, nucleases, and other contaminants or inhibitors.

If processing stool material, the performance of a vacuum check is recommended. The maximum recommended starting material for stool samples is 200 mg; for dehydrated stool samples, start with no more than 100 mg.

Microbial genomic DNA purified using the DNeasy 96 PowerSoil Pro QIAcube HT Kit is ready for use in enzymatic reactions, such as PCR, or it can be stored at -30 to -15°C .

Bead beating

The DNeasy 96 PowerSoil Pro QIAcube HT Kit is designed to be used in combination with either PowerBead Pro Plates (cat. no. 19311) or PowerBead Pro Tubes (cat. no. 19301). The use of other disruption media can lead to reduced yields of microbial genomic DNA.

For convenient high-throughput 96-well homogenization, we offer the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990). In conjunction with PowerBead Pro Plates, the TissueLyser II provides high-throughput processing for simultaneous, rapid, and effective disruption of up to 2×96 samples in only a few minutes.

For disruption using 2 ml PowerBead Pro Tubes, the TissueLyser II provides simultaneous disruption of up to 48 samples in combination with the TissueLyser Adapter Set 2×24 (cat. no. 69982) or up to 96 samples in combination with the 2 ml Tube Holder and Plate Adapter Set.

Use of the PowerLyzer 24 Homogenizer (cat. no. 13155) allows the simultaneous disruption of up to 24 samples.

However, since the DNeasy 96 PowerSoil Pro QIAcube HT Kit does not necessarily require high-velocity bead beating, homogenization using a Vortex-Genie[®] 2 with Vortex Adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24) can be used for disruption in conjunction with PowerBead Pro Tubes.

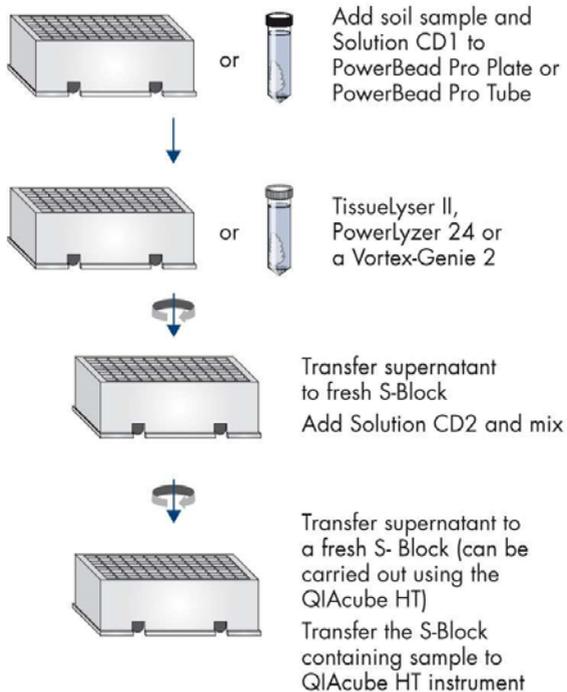


Figure 1. DNeasy 96 PowerSoil Pro QIAcube HT Kit workflow. Sample lysis and inhibitor removal.

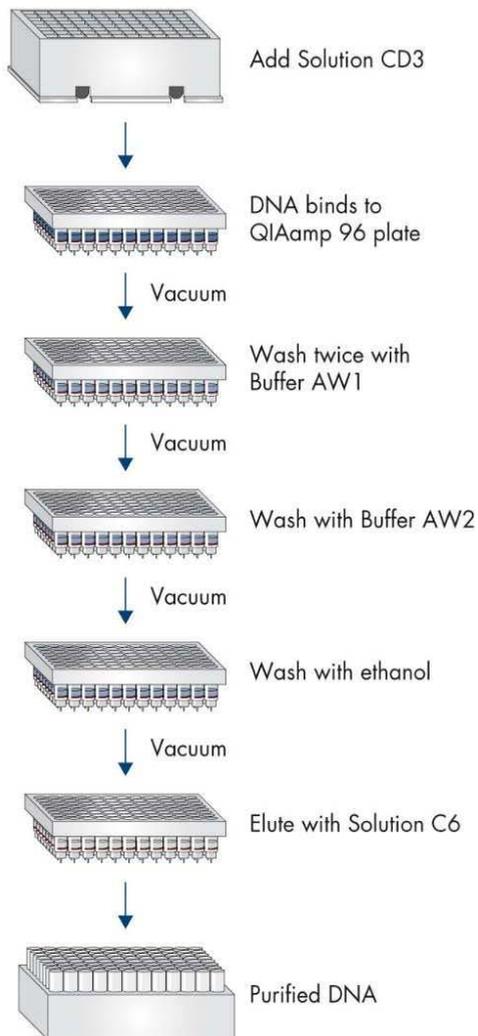


Figure 2. DNeasy 96 PowerSoil Pro QIAcube HT Kit workflow.
QIAcube HT DNeasy 96 PowerSoil Pro Protocol.

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.

- Pipettes and disposable pipette tips with aerosol barriers (20–1000 μ l)
- Ethanol (96–100%)*
- QIAcube HT Instrument
- QIAcube HT Prep Manager Software
- QIAcube HT Reagent troughs
- QIAcube HT Plasticware (cat. no. 950067)
- Disposable gloves
- PowerBead Pro Tubes or PowerBead Pro Plates for bead-beating homogenization
- Centrifuge with Plate Rotor 2 x 96 (up to 4500 x *g*) or a microcentrifuge (up to 15,000 x *g*)
- High-velocity bead beater such as the TissueLyser II with corresponding adapter sets for use with PowerBead Pro Plates [Plate Adapter Set (cat. no. 11990)] or PowerBead Pro Tubes [TissueLyser Adapter Set 2 x 24, 2 ml Tube Holder, and Plate Adapter Set]
Alternately, for use with PowerBead Pro Tubes, a Vortex-Genie 2 with Vortex Adapter for 24 (1.5–2 ml) tubes or the PowerLyzer 24 Homogenizer (cat. no. 13155)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important Notes

Processing fewer than 96 samples per run

If processing fewer than 96 samples, you can reuse the unused portions of the QIAamp 96 Plates, S-Blocks, and Elution Microtubes RS up to 3 times.

We recommend using fresh plasticware for every run. If reusing, take extreme care to prevent cross-contamination.

- At a minimum, process at least 24 samples next to each other
- Process samples together in increments of 8
- Cover unused wells of the S-Block and QIAamp 96 Plate with adhesive tape
- Store plates to ensure separation of the outlet nozzles under the plate – in S-Blocks used in the same run or in a fresh 96-well microtiter plate, for example

Preparing reagents

Buffer AW1 and Buffer AW2

Buffer AW1 and Buffer AW2 are supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as indicated on the bottles to obtain a working solution. Tick the check box on the bottle labels to indicate that ethanol has been added. Mix well after adding ethanol.

TopElute Fluid

TopElute Fluid is used during elution of nucleic acids from the QIAamp 96 Plate membranes. It enables application of a stable and high vacuum and results in equal eluate volumes. In addition, TopElute Fluid eliminates the formation of drops of elution buffer at the outlet nozzles of the QIAamp 96 Plates.

TopElute Fluid might be found as a top layer over the eluted sample. It is inert and has no effects on downstream applications.

Important: Please ensure that you only take the eluate from below the top layer.

Assembling the vacuum chamber

Figure 3 illustrates the assembly of the vacuum chamber. For further information, please refer to the *QIAcube HT User Manual*.

- Insert the channeling block holder into the left (waste) chamber of the vacuum chamber.
- Press firmly on the sides of the channeling block holder to seat it in the chamber, sealing the O-ring on the spigot into the drain.
- Place the channeling block into the channeling block holder.
- Place the QIAamp 96 Plate in the transfer carriage. Load the carriage with the QIAamp 96 Plate into the left (waste) chamber of the vacuum chamber.
- Ensure that the carriage is positioned to the left inside the vacuum chamber. Place the riser block EMTR in the right (elution) chamber of the vacuum chamber, with the pin of the riser block EMTR in top-right position. Load EMTR (without lid) into elution chamber.

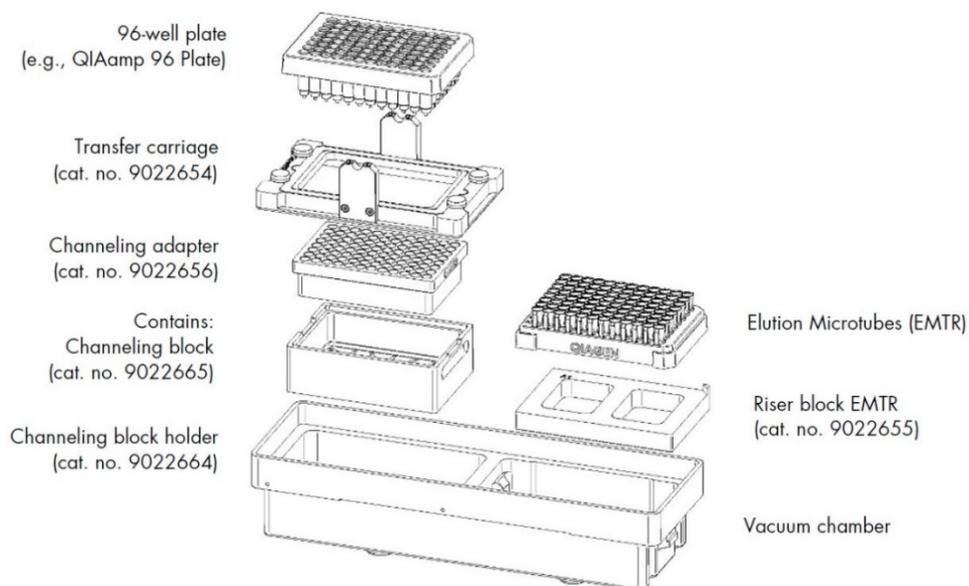


Figure 3. Assembling the vacuum chamber.

Protocol: Preparing Samples

Important notes before starting

- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Make sure Buffer AW1 and AW2 are prepared according to the instructions on the bottles.
- All procedure steps, including all centrifugation steps, should be performed at room temperature (15–25°C). Avoid any interruptions.
- Ensure that the relevant version of the DNeasy 96 PowerSoil Pro QIAcube HT Kit Configuration XML files (available from www.qiagen.com/DNeasy96PowerSoilProQIAcubeHTKit/Resources > **Operating Software**), which contains all the information required to process DNeasy 96 PowerSoil Pro QIAcube HT kit on the QIAcube HT instrument, is configured to the QIAcube HT Prep Manager Software.
- QIAcube HT Prep Manager kit configuration XML files, which contain all the information required to perform a run on the QIAcube HT instrument, are available from www.qiagen.com/QIAcubeHT-Resources.
- Ensure that QIAcube HT Prep Manager Software is installed in your computer.
- Ensure that you are familiar with the procedures for operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.
- For lysis and disruption, use either PowerBead Pro Plates or PowerBead Pro Tubes. For more detailed information, please see the “Bead beating” section in the Introduction, on page 8.

Procedure

1. Spin the PowerBead Pro Plate or the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Remove the square well mat from the PowerBead Pro Plate or open the PowerBead Pro Tube.
2. Add 800 µl Solution CD1 with up to 250 mg of soil or up to 200 mg of stool. Seal the plate or recap the tube, and vortex briefly to mix.

Note: After your sample has been loaded into the PowerBead Pro Plate or PowerBead Pro Tube, the next step is a homogenization and lysis procedure. The plate or tube contains a buffer that will (a) help disperse the soil or stool particles, (b) begin to dissolve humic acids and other inhibitors, and (c) protect nucleic acids from degradation. Gentle vortexing mixes the components in the plate and begins to disperse the sample in the buffer.

3. Homogenize samples thoroughly using one of these methods:

○ **A. TissueLyser II:**

Place the sealed PowerBead Pro Plate between 2 adapter plates (cat. no. 11990). Shake for 5 min at speed 25 Hz. Reorient the plates so that the side that was closest to the machine body is now furthest. Shake again for 5 min at speed 25 Hz.

Place the PowerBead Pro Tube into a TissueLyser Adapter Set 2 x 24 or a 2 ml Tube Holder and Plate Adapter Set (cat. no. 11990). Fasten the adapter into the instrument and shake for 5 min at speed 25 Hz. Reorient the adapter so that the side that was closest to the machine body is now furthest. Shake again for 5 min at speed 25 Hz.

○ **B. PowerLyzer 24:**

Place Power Bead Pro Tubes symmetrically into the tube holder and secure the finger plate in place over the carousel. Operate for 2 x 30 s with a 30 s break at 2000–4000 rpm.

Note: Higher forces bear the risk of stronger fragmentation but give higher DNA yields.

○ **C. Vortex-Genie 2:**

Secure PowerBead Pro Tubes horizontally using a Vortex Adapter for 24 (1.5–2.0 ml) tubes. Vortex at maximum speed for 10 min.

Note: If using the 24-place Vortex Adapter for more than 12 preps, increase the vortexing time by 5–10 min. Avoid using tape, which can become loose and result in reduced homogenization efficiency, inconsistent results, and lower yields.

Important: Step 3 is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents (from step 2) and mechanical shaking during this

step. Randomly shaking the beads in the presence of disrupting agents promotes collision between beads and microbial cells, causing the cells to lyse.

4. Centrifuge the PowerBead Pro Plate at 4500 x g for 6 min or the PowerBead Pro Tube at 15,000 x g for 1 min.
5. Transfer the supernatant to a new S-Block (provided).

Note: Expect 480–600 µl. The supernatant may still contain some particles.

6. Add 250 µl Solution CD2 and mix by pipetting. Seal plate carefully with sealing tape.

Note: Solution CD2 contains Inhibitor Removal Technology (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 to use 300 µl Solution CD2

7. Centrifuge at 4500 x g for 6 min at room temperature.
8. Avoiding the pellet, transfer 550 µl of supernatant to a fresh S-Block either manually or automatically using the QIAcube HT.

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.

Note: After manual transfer, proceed with the DNeasy 96 PowerSoil Pro Protocol on the QIAcube HT. Transfer S-Block containing the sample to the QIAcube HT instrument.

Note: For automated transfer, see “Transfer Protocol Using the QIAcube HT”. Transfer the S-Block from step 7 to the QIAcube HT instrument. After automated transfer, proceed with the DNeasy 96 PowerSoil Pro Protocol on the QIAcube HT.

DNeasy 96 PowerSoil Pro Protocol on the QIAcube HT

Procedure

1. Switch on the QIAcube HT instrument and the connected laptop computer. We recommend leaving the vacuum station switched on at all times.
2. Launch the QIAcube HT Prep Manager Software.
3. On the Home screen, select the **DNeasy 96 PowerSoil Pro** experiment.
4. Enter the kit information.
5. Select the type of sample to be processed. Select **soil**, **stool**, or **any** when running the DNeasy 96 PowerSoil Pro Protocol.
6. Select the pretreatment from the drop-down menu. For the DNeasy 96 PowerSoil Pro Protocol, the pretreatment is **manual**.
7. Select the protocol: **DNeasy 96 PowerSoil Pro Protocol**.

Optional: Set optional steps for checking vacuum performance.

Note: The run script provided by QIAGEN includes all necessary steps to guarantee best performance. Nevertheless, it is possible to check the vacuum performance during the run, if desired. If this option is selected, the instrument will pause after the binding step, so you can check if any wells may be clogged. The vacuum check is recommended for stool samples. For more information, see “Vacuum performance check”, page 25.

8. Click **Next**.

Optional: Click **Save**. With the QIAcube HT Prep Manager software, it is possible to save an experiment at any step in the run setup procedure.

9. In the “Labware Selection” screen, select one of the options below:
 - A. Choose **Use sample IDs or existing sample input file** and load existing sample file if you want to load your samples from an existing file.

B. Check **Auto-generate sample IDs** for all samples to automatically generate sample IDs.

C. Press **Define samples** to start naming the samples manually. For more information about creating sample IDs, refer to the *QIAcube HT User Manual*.

Note: We do not recommend changing the input labware.

10. In the "Plate Assignment" screen, select the samples from the input and assign them to the output plate. Click **Next**.

Note: The input plate is set according to your definition in the "Labware Selection" screen. Select the sample columns on the input labware, and then click **Assign** to assign them to the output plate.

It is possible to change the assignment, if required. Select the columns on the output labware and click **Remove assignment**. Click and hold the left mouse button to mark the columns and rearrange them as needed.

Note: The instrument must be switched on and connected to the software before entering the "Worktable" step.

Note: We recommend using a minimum of 24 samples. Arrange the samples without any gaps.

11. In the "Worktable setup" screen, select any virtual worktable position marked in yellow and follow the instructions to set up the instrument worktable.

Important: All positions to be used on the worktable must be calibrated. If the "Calibrate Labware" button appears yellow, calibration is required. For further information, see the *QIAcube HT User Manual*.

12. Ensure that the liquid level in the waste bottle matches the indicated level on the virtual desktop. The level is displayed by a color code:

- Green = no action is necessary
- Yellow = the bottle is almost full but the run can be started
- Red = the bottle needs to be emptied before starting the run

13. Place the tip chute on the worktable so that the chute is over the tip-disposal box. Ensure that the tip chute is open and unblocked.

14. Remove the UV protective cap from the tip chute. Ensure that the tip-disposal box is empty and that the opening is aligned with the tip chute.
15. Prepare the vacuum chamber as shown in Figure 3 (page 13). The assembly is also described in the *QIAcube HT User Manual*.

Important: If fewer than 12 columns (96 wells) are to be processed, seal the unused columns of the QIAamp 96 Plate with adhesive tape. Unused wells must be sealed to ensure proper vacuum operation. Process a minimum of 24 samples per run.

Note: Trim any excess tape.

Note: When reusing a QIAamp 96 Plate, S-Block, or elution plate, take care to avoid cross-contamination.

Note: Make sure the QIAamp 96 Plate is aligned to the left in the carriage and that the carriage is positioned to the left inside the vacuum chamber.

16. Load the tips in the indicated positions and remove the lids from the tip boxes. Ensure that there are sufficient numbers of tips for all steps, at least up to and including the lysate transfer step.

Note: Check that the number and position of available and unused tips on the instrument worktable match those of the displayed tips on the virtual worktable. If more tips are required, you will be prompted to replace empty tip racks with new tip racks during the run.

17. Transfer the indicated volumes of all reagents into the corresponding reagent troughs. Close the lids and place the troughs in the indicated positions on the worktable.
18. Place the QIAGEN S-Block containing the samples (from "Protocol: Preparing Samples" step 8, page 16) on the indicated position of the QIAcube HT worktable (sample volume is 550 µl).

Note: Samples are processed in columns of 8 wells. We recommend covering unused wells during pipetting so that unused S-Block wells can be used in subsequent runs.

Note: If fewer than 8 samples in a column are to be processed, add water or buffer to the unused wells in the column. To avoid foaming, the volume of water or buffer added to the unused wells should be the same as the sample volumes.

19. Close the instrument hood and click **Start run**. The protocol run will begin.

Important: At the beginning of each run, an open circuit test and a plate detection test will be performed automatically. If the QIAamp 96 Plate in the transfer carriage is improperly aligned, you will be prompted to position it correctly to the left compartment of the vacuum chamber. After correcting the plate's position, click **Retry** to initiate the tests again. For more information about the system tests, see the *QIAcube HT User Manual*.

Optional: If you chose to use a vacuum performance check, the protocol stops for a manual interaction after the binding step.

20. After the protocol is finished, create a report if necessary.

21. Cover the elution microtubes (EMTR) with the lid and remove from the elution chamber.

Note: We recommend storing the DNA frozen (-30 to -15°C or -90 to -65°C , for standard freezers set at -20°C or -80°C , respectively) because Solution C6 does not contain EDTA. To concentrate DNA, refer to the "Troubleshooting Guide" on page 30.

Note: Two liquid phases may be found in the elution microtubes. If this is the case, TopElute Fluid will be found as a top layer over the eluted sample. It is inert and has no effect on downstream applications.

Important: Please ensure that you only take the eluate from below the top layer for downstream applications.

22. Proceed to "Cleaning the Instrument After Completing a Run" on page 24.

Transfer Protocol Using the QIAcube HT

This protocol is for the automated transfer of 550 µl of supernatant into a fresh S-Block on the QIAcube HT after samples have been treated with Solution CD2 and centrifuged (after step 7 of the Preparing Samples protocol, page 16).

This is a standard protocol that is able to handle most sample types. However, if sample materials are processed that generate lower volumes of supernatant after disruption than generally expected (less than 480 µl), the transfer protocol might pipet sample volumes lower than 550µl resulting in slightly decreased yields. For these instances, please request a customized version of the transfer protocol by contacting QIAGEN Technical Services at support.qiagen.com.

Important notes before starting

- Ensure that the relevant version of the DNeasy 96 PowerSoil Pro QIAcube HT Kit Configuration XML files (available from www.qiagen.com/DNeasy96PowerSoilProQIAcubeHTKit/Resources > **Operating Software**), which contains all the information required to process DNeasy 96 PowerSoil Pro QIAcube HT kit on the QIAcube HT instrument, is configured to the QIAcube HT Prep Manager Software.
- QIAcube HT Prep Manager kit configuration XML files, which contain all the information required to perform a run on the QIAcube HT instrument, are available from www.qiagen.com/QIAcubeHT-Resources.
- Ensure that QIAcube HT Prep Manager Software is installed.
- Ensure that you are familiar with the operation of the instrument. Please refer to the *QIAcube HT User Manual* for operating instructions.

Procedure

1. Switch on the QIAcube HT instrument and the connected laptop computer. We recommend leaving the vacuum station switched on at all times.

2. Launch the QIAcube HT Prep Manager Software.

Important: When using the protocol for the first time, the QIAGEN S-Block has to be made available under the “Labware Manager” option of the QIAcube HT Prep Manager software. For further information about the Labware Manager, please see the *QIAcube HT User Manual*.

3. On the **Home** screen, select the **DNeasy 96 PowerSoil Pro Experiment**.

4. Enter the kit information.

5. Select the type of sample to be processed. Select **soil**, **stool** or **any** when running the DNeasy 96 PowerSoil Pro Protocol

6. Select the pretreatment from the drop-down menu. For the DNeasy 96 PowerSoil Protocol, the pretreatment is **Manual**. Select the protocol: **Transfer Protocol**.

7. Click **Next**.

Optional: Click **Save**. With the QIAcube HT Prep Manager software, it is possible to save an experiment at any step in the run setup procedure.

8. In the “Labware Selection” screen, select one of the options below:

A. Choose **Use sample IDs or existing sample input file** and load existing sample file, if you want to load your samples from an existing file.

B. Check **Auto-generate sample IDs** for all samples to automatically generate sample IDs.

C. Press **Define samples** to start naming the samples manually. For more information about creating sample IDs, refer to the *QIAcube HT User Manual*.

Note: We do not recommend changing the input labware.

9. In the “Plate Assignment” screen, select the samples from the input and assign them to the output plate. Click **Next**.

Note: The input plate is set according to your definition in the “Labware Selection” screen. Select the sample columns on the input labware and then click **Assign** to assign them to the output plate.

It is possible to change the assignment, if required. Select the columns on the output labware and click **Remove assignment**. Click and hold the left mouse button to mark the columns and rearrange them as needed.

Note: The instrument must be switched on and connected to the software before entering the Worktable step.

10. In the "Worktable setup" screen, select any virtual worktable position marked in yellow and follow the instructions to set up the instrument worktable.

Important: All positions to be used on the worktable must be calibrated. If the **Calibrate Labware** button appears yellow, calibration is required. For further information, see the *QIAcube HT User Manual*.

11. Ensure that the liquid level in the waste bottle matches the indicated level on the virtual desktop. The level is displayed by a color code:
 - Green = no action is necessary
 - Yellow = the bottle is almost full but the run can be started
 - Red = the bottle needs to be emptied before starting the run
12. Place the tip chute on the worktable so that the chute is over the tip disposal box. Ensure that the tip chute is open and unblocked.
13. Remove the UV protective cap from the tip chute. Ensure that the tip disposal box is empty and that the opening is aligned with the tip chute.
14. Load the tips in the indicated positions and remove the lids from the tip boxes. Ensure that there are sufficient tips for all the steps.
15. Place the QIAGEN S-Block containing the centrifuged samples from step 7 of "Protocol: Preparing Samples" (page 14) and a new S-Block on the indicated positions of the QIAcube HT worktable.

Note: The virtual worktable setup screen requires 700 µl sample volume in the S-Block, which is assumed to be the volume of the supernatant. However, it is neither necessary to measure the supernatant nor fill it up to 700 µl.

16. Close the instrument hood and click **Start run**. The protocol run will begin.
After the run, remove the first S-Block and keep the second S-Block containing the 550 µl supernatant. Proceed to the "DNeasy 96 PowerSoil Pro Protocol on the QIAcube HT".

Cleaning the Instrument after Completing a Run

Procedure

1. Follow the instructions in the QIAcube HT Prep Manager Software for cleaning the instrument after a run.
2. Cover tip racks that contain only unused tips with lids, and remove them from the worktable.
3. Cover fractions of partly used tip racks with adhesive tape. And then, cover the tip racks with lids and remove from the worktable. Discard empty tip racks.
4. If the run was stopped and the instrument did not remove all the used tips, remove them now and discard them.
5. Remove all reagent troughs and discard them.
Note: Do not reuse reagents for multiple runs.
6. Remove the input plate.
7. Discard the QIAamp 96 Plate or keep partially used QIAamp 96 Plates for subsequent reuse. (In case of the latter, cover used fractions with adhesive tape.)
8. Remove the tip chute and all adapters from the worktable. Remove the carriage, channeling adapter, and riser block from the vacuum chamber. Clean all parts as described in the *QIAcube HT User Manual*.
9. Discard the tip disposal box.
10. Use a damp cloth to clean any reagents that may have spilled onto the instrument worktable or vacuum chamber.
11. Discard all waste according to local safety regulations.

Note: For detailed instructions on all further cleaning and maintenance operations, please see the *QIAcube HT User Manual*.

Optional Step: Vacuum Performance Check

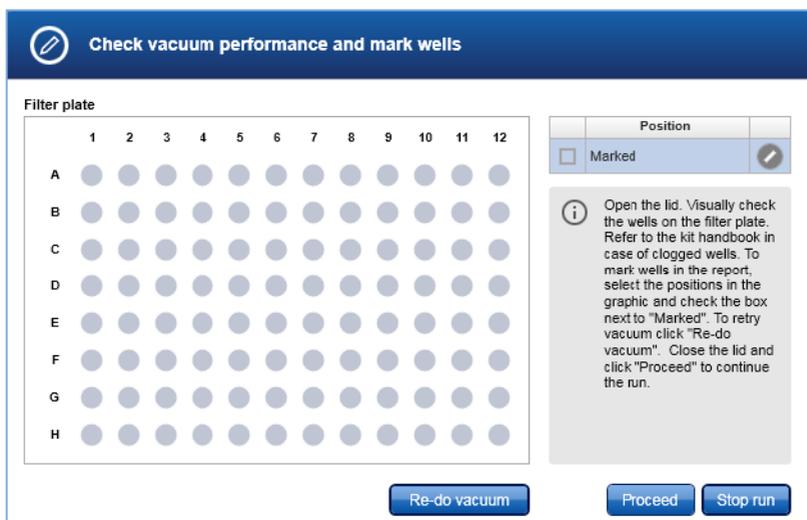
Selecting the “Vacuum performance check after binding step,...” option results in one manual interaction pause after the binding step:

Optional Steps (for details refer to kit handbook)



This optional setting allows the user to check whether all of the liquid has passed through the membranes. By default, this step is not checked. We recommend using the vacuum performance check when processing stool samples.

If the vacuum performance check step is activated (as in the screenshot above), the instrument will pause after the binding step. You can then check to see if all liquid has passed through the membranes, and decide whether to reapply the vacuum (**Re-do vacuum**) or continue with the procedure (**Proceed**).



Procedure

1. Open the instrument lid.

Note: The lid sensor is disabled during the vacuum performance check, allowing the user to observe the wells.

2. Check the wells on the QIAamp 96 Plate for any remaining liquid. If no liquid is visible in the wells after the vacuum step, click **Proceed** to continue the run. If liquid remains in the wells, click **Re-do vacuum** to apply vacuum again. The vacuum will be activated for a certain period of time or until **Stop vacuum** is pressed.

3. Mark any well that is clogged or not empty in the dialog that appears. This information will be included in the run report. To mark a well, select the position in the dialog.

Note: To select multiple positions, press the **Shift** key and left-click with the mouse to select adjacent positions, or press the **Ctrl** key and left-click with the mouse to select nonadjacent positions. Alternatively, drag the mouse to select adjacent positions in a rectangle.

4. In the position table at the right, check the box next to **Marked**. The selected position on the QIAamp 96 plate will be displayed as marked.

Note: To unmark a position, select the position and uncheck the box next to **Marked**.

5. If liquid still remains in any well, manually remove the liquid using a pipette.

Check vacuum performance and mark wells

Filter plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	●	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●
D	●	●	●	●	●	●	●	●	●	●	●	●
E	●	●	●	●	●	●	●	●	●	●	●	●
F	●	●	●	●	●	●	●	●	●	●	●	●
G	●	●	●	●	●	●	●	●	●	●	●	●
H	●	●	●	●	●	●	●	●	●	●	●	●

Position
<input checked="" type="checkbox"/> Marked

i Open the lid. Visually check the wells on the filter plate. Refer to the kit handbook in case of clogged wells. To mark wells in the report, select the positions in the graphic and check the box next to "Marked". To retry vacuum click "Re-do vacuum". Close the lid and click "Proceed" to continue the run.

Re-do vacuum Proceed Stop run

-
6. After the instrument has added additional reagents, open the hood to pause the run.
Check to see whether the affected well is still blocked. If so, manually remove the liquid from the affected well using a pipette.
 7. Click either **Proceed** to continue or **Stop run** to stop.

Advanced Options

Important: QIAGEN does not recommend modifying any of the parameters found in the “Advanced options” section. These parameters have been optimized for each QIAcube HT Kit to guarantee accurate and valid experiment results. Please note that any changes to these options are carried out at your own risk.

Note: A warning icon and a corresponding warning message will be displayed if you change any parameter. The warning text contains the recommended value. If you return to the recommended value, the warning message will disappear.

Advanced options

Vacuum parameter

Vacuum intensity	Vacuum time
<input type="text" value="60"/> kPa	<input type="text" value="240"/> sec

Elution parameter

Total elution volume	Elution steps	<input checked="" type="checkbox"/> Top elute
<input type="text" value="120"/> µl [80 - 120 µl]	<input type="text" value="1"/> ▼	

Vacuum parameters

In the “Vacuum parameter” section, it is possible to change 2 parameters: vacuum intensity and vacuum time. The default settings are 60 kPa for vacuum intensity and 240 s for vacuum time.

The vacuum intensity can be changed from 60 kPa to 70 kPa, and the vacuum time can be changed from 60 s to 480 s. These changes are *not* recommended by QIAGEN.

Note: If you change the vacuum intensity or vacuum time parameter, it only affects the vacuum intensity and/or vacuum time following the binding step. All other vacuum steps will be unaffected.

Elution parameter

In the “Elution parameter” section, it is possible to change the total elution volume. The recommended values for the elution parameters are included in the QIAcube HT Prep Manager Software. The total elution volume can be changed to a value within the defined parameter range.

TopElute Fluid

TopElute Fluid is used during elution of nucleic acids from the QIAamp membrane. It enables application of a stable and high vacuum and results in equal eluate volumes. In addition, TopElute Fluid eliminates the formation of drops of elution buffer at the outlet nozzles of the QIAamp 96 Plates.

By default, the Top Elute option is checked. If TopElute Fluid should not be used during the run, uncheck **TopElute** under Advanced options.

Important: We do not recommend changing the use of TopElute Fluid, as that has not been tested by QIAGEN.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies. For contact information, visit www.qiagen.com.

Comments and suggestions

Soil processing

- | | |
|---------------------------------------|--|
| a) Amount of soil or stool to process | The QIAGEN DNeasy 96 PowerSoil Pro QIAcube HT Kit is designed to process 0.25 g of soil or 0.2 g of stool. For inquiries regarding the use of larger sample amounts, please contact Technical Support for suggestions. |
|---------------------------------------|--|

DNA

- | | |
|--|---|
| a) DNA does not amplify | 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 that was isolated using the QIAGEN PowerSoil Pro DNA 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 Technical Support for suggestions. |
| c) DNA floats out of a well when loading a gel | This usually occurs because residual ethanol remains in the final sample. Ethanol precipitation (described in “Concentrating eluted DNA”, page 30) is the best way to remove residual Solution C5. |
| d) 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 × <i>g</i> for 5 min at room temperature. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid overdrying the pellet, or resuspension may become difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris (Solution C6). |

- e) Storing DNA DNA is eluted in Solution C6 (10 mM Tris) and must be stored at -30 to -15°C or at -65 to -90°C (depending on freezer settings) 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 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 for 3–4 s, and then heat to 70°C for 5 min. Repeat once. This alternative procedure will reduce shearing but may also reduce yield.

Instrument issues

- a) Recovery in case of user interruption Runs on the QIAcube HT get interrupted if the hood is opened. The run will proceed normally once the hood is closed. To ensure process safety, this incident is reported in the post-run report.
- b) Blocked membranes When processing samples that might block the membranes, we recommend using a vacuum performance check.
- If liquid is still visible, remove it using a pipet, and then scrape the surface of the membrane with a fresh pipette tip to relocate any solid particles that may block the membrane. Take care not to damage the membrane. If there is still no liquid flow, pipet all liquid from the well and proceed with the run.
- After the instrument has added wash buffer, open the hood to pause the run. Check if the well is still blocked. If so, remove all liquid using a pipette.
- Do not perforate the membrane. Uncovered perforated wells will disturb vacuum integrity across the whole plate during elution.
- Proceed with the run, and use less sample next time.
- c) Instrument failure/cancelled run It is not possible to recover the run. Continue manually.

Ordering Information

Product	Contents	Cat. no.
DNeasy 96 PowerSoil Pro QIAcube HT Kit	For 480 preps: Isolation of microbial genomic DNA from all soil types on the QIAcube HT	47021
QIAcube HT Plasticware	Plasticware for 480 typical preps on the QIAcube HT: 5 S-Blocks, 5 Elution Microtubes RS (EMTR), 2 x 50 caps for EMTR, 9 x 96 Filter-Tips OnCor C, tape pad	950067
Elution Microtubes RS	EMTR for use with QIAcube HT: 24 x 96 elution microtubes in racks of 96; includes strip caps	120008
S-Blocks	For collecting wash and lysis fractions from 96-well plates	19585
Filter-Tips, OnCor C, 200 µl (10 x 96)	Disposable filter-tips; for use with QIAcube HT	990610
Reagent Trough (with lid), 70 ml	Box of 10 plus lid; liquid reservoirs; used with Reagent Trough R1 Position Plate and Reagent Trough SBS Plate	990554
Reagent Trough (with lid), 170 ml	Box of 10 plus lid; liquid reservoirs; used with Reagent Trough R1 Position Plate and Reagent Trough SBS Plate	990556
Tip Disposal Box	Box of 10; plastic waste collection box with secure-fitting lid to fit tip ejector chute	990550
TopElute Fluid (60 ml)	For use with automated nucleic acid purification protocols	1055628

Product	Contents	Cat. no.
PowerBead Pro Plates (4)	For high-throughput disruption of all soil types and stool samples in 96-well format	19311
PowerBead Pro Tubes	For disruption of all soil and stool samples in 2 ml format	19301
Related products		
TissueLyser II*	For medium- to high-throughput sample disruption for molecular analysis	85300
TissueLyser Adapter Set 2 x 24	For efficient disruption of up to 48 samples in parallel on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	For efficient disruption of up to 192 samples in parallel on the TissueLyser II	69984
2 ml Tube Holder Set	For the homogenization in 2 ml bead tubes on the 96-well plate shaker	11993
TissueLyser LT	For low- to medium-throughput sample disruption for molecular analysis†	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Vortex Adapter	For vortexing 1.5 ml or 2 ml tubes using the Vortex-Genie 2	13000-V1-24
DNeasy PowerMax® Soil Kit (10)	For 10 preps: Isolate microbial DNA from large quantities of soil; great for samples with low microbial load	12988-10

* The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or the TissueLyser Adapter Set 2 x 96 (cat. no. 69984).

† The TissueLyser LT must be used in combination with the TissueLyser LT Adapter, 12-Tube.

Product	Contents	Cat. no.
RNeasy® PowerSoil Total RNA Kit (25)	For 25 preps: Isolate high-quality total RNA from all soil types	12866-25
MagAttract® PowerSoil DNA KF Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27000-4-KF
MagAttract PowerSoil DNA EP Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27100-4-EP
DNeasy PowerSoil Pro Kit (50)	For 50 preps: Isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For 250 preps: Isolation of microbial genomic DNA from all soil types	47016
QIAamp PowerFecal® Pro DNA Kit (50)	For 50 preps: Isolation of microbial DNA from stool, gut material, and biosolids	51804

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

Revision History

Date	Changes
08/2019	Updated storage conditions. Corrected listed cat. no. for Plate Adapter Set. Added details for finding the DNeasy 96 PowerSoil Pro QIAcube HT Kit Configuration XML files in "Important notes before starting". Simplified instructions leading to the QIAcube HT Prep Manager kit configuration XML files (old: " www.qiagen.com/QIAcubeHT ", under the Resources tab"; new: " www.qiagen.com/QIAcubeHT-Resources ").

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