September 2016

# QIAamp® 96 PowerFecal QIAcube® HT Handbook

For automated purification of total DNA from human stool samples using QIAcube HT and QIAcube HT Prep Manager Software

Sample to Insight

QIAGEN

# Contents

# Kit Contents

| **QIAamp 96 PowerFecal QIAcube HT Kit** | **(5)** |
|---|---|
| **Catalog no.** | **51531** |
| **Number of preps** | **480** |
| QIAamp 96 plates | 5 |
| Buffer PW1* | 6 x 55 ml |
| Buffer C3 | 2 x 44 ml |
| Buffer C4* | 1 x 330 ml |
| QIAGEN® Proteinase K | 2 x 6 ml |
| Buffer AW1*† (concentrate) | 1 x 242 ml |
| Buffer AW2† (concentrate) | 1 x 127 ml |
| Buffer ATE | 2 x 45 ml |
| TopElute Fluid | 60 ml |
| 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 6 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 |

The following components can also be ordered separately: S-Blocks, Elution Microtubes RS (including caps for strips) and TopElute Fluid. See "Ordering Information", page 32.

# Storage

QIAamp 96 plates and all buffers are stable at room temperature (15–25°C) and dry conditions until the expiration date on the kit box without affecting performance.

QIAGEN Proteinase K can be stored at room temperature (15–25°C). To store for extended periods of time, or if the ambient temperature often exceeds 25°C, we recommend storing at 2–8°C.

# Intended Use

The QIAamp 96 PowerFecal QIAcube HT Kit is intended for the automated extraction of DNA using the QIAcube HT instrument. The QIAamp 96 PowerFecal QIAcube HT Kit is intended for molecular biology applications. This product is 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.

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

Buffer C4 and Buffer AW1 contain guanidine hydrochloride and Buffer PW1 contains guanidine thiocyanate, which can form highly reactive compounds if combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled 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 QIAamp 96 PowerFecal QIAcube HT Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

The QIAamp 96 PowerFecal QIAcube HT Kit uses well-established technology for purification of total DNA from fresh or frozen stool samples. The procedure yields high-quality DNA that performs well in PCR and other enzymatic reactions.

The QIAamp 96 PowerFecal QIAcube HT Kit combines the selective binding properties of silica-based membrane with a high-throughput 96-well format, and is designed for fully automated, simultaneous processing of 24–96 samples of 200 mg stool, on the QIAcube HT instrument system (in increments of 8). Purification requires no phenol-chloroform extraction or alcohol precipitation and involves minimal handling. DNA is eluted in low-salt buffer, and is free of proteins, nucleases and other contaminants or inhibitors. The purified DNA is ready for use in enzymatic reactions, such as PCR, or storage at –15 to –30°C.

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure removal of these substances, the QIAamp 96 PowerFecal QIAcube HT Kit contains IRT (Inhibitor Removal Technology), which is specially formulated to separate inhibitory substances from DNA in stool samples.

## Principle and procedure

The QIAamp 96 PowerFecal QIAcube HT procedure is simple (see flowchart on page 11). Samples are first mechanically lysed. Subsequently impurities are separated from stool samples using patented Inhibitor Removal technology. Buffering conditions are adjusted to provide optimal DNA binding conditions, and the lysate is loaded onto the QIAamp 96 plate. During vacuum, DNA is selectively bound to the QIAamp membrane. Remaining contaminants and enzyme inhibitors are removed in four efficient wash steps. Pure DNA is eluted under vacuum in a single step in approximately 100 µl of Buffer ATE equilibrated to room temperature (15–25°C). DNA recovery is enhanced by overlaying the elution buffer with TopElute Fluid.

## Sample size

The QIAamp 96 PowerFecal QIAcube HT Kit is optimized for use with up to 200 mg fresh or frozen stool. If working with dehydrated or dry stool samples, resuspend the sample in Buffer PM1 to obtain the necessary amount of supernatant after the first precipitation step. For maximum flexibility, manual sample preparation can be carried out in either 2 ml microcentrifuge tubes or 96-well plates.

## Lysis and removal of impurities using inhibitor removal technology

In the first steps of the protocol, stool samples are mechanically lysed in Buffer PW1. This is recommended for disruption of cells that are difficult to lyse (e.g., some bacteria and parasites). After lysis, DNA-degrading substances and PCR inhibitors present in the stool sample are separated from the DNA by precipitation using Buffer C3. The DNA in the supernatant is purified on a QIAamp 96 plate.

## Purification on QIAamp 96-well plates

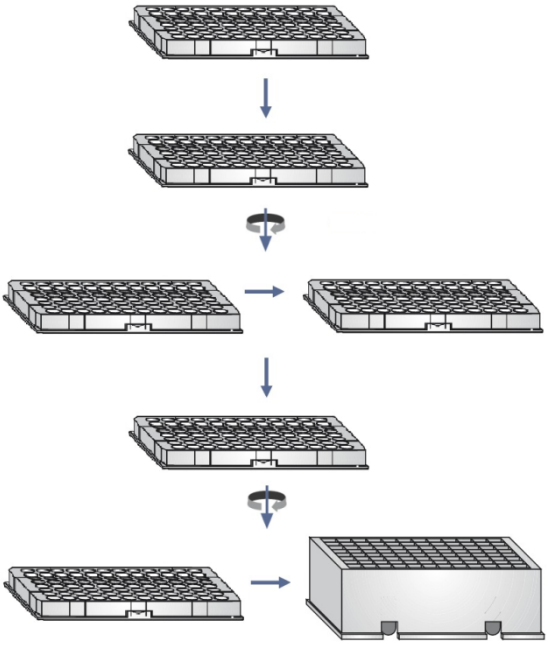
The QIAamp DNA purification procedure involves digestion of proteins, binding of DNA to the QIAamp silica membrane, washing away impurities, and eluting pure DNA from the plate. Proteins are digested and degraded under denaturing conditions with proteinase K. Buffering conditions are then adjusted to allow optimal binding of DNA to the QIAamp membrane, and the sample is loaded onto the QIAamp 96-well plate.

DNA is adsorbed onto the QIAamp silica membrane during a vacuum step. After the vacuum step an optional user confirmation step is implemented by default in the protocol to guard against clogging issues. If wells still contain lysate, the vacuum step can be prolonged. If clogging still remains, residual lysate needs be removed manually before the protocol can proceed.

Optimized wash conditions using two wash buffers ensure complete removal of any residual impurities without affecting DNA binding. Purified, concentrated DNA is eluted from the QIAamp 96-well plate in low-salt buffer equilibrated to room temperature. DNA yield is

typically 10–50 µg but, depending on the individual stool sample and the way it was stored, may range from 5–100 µg. DNA concentration is typically 50–250 ng/µl. The eluted DNA is suitable for direct use in PCR and other enzymatic reactions. If the purified DNA is to be kept, storage at –15 to –30°C is recommended.

Sample lysis and homogenization



Add stool sample and Buffer PW1 to 96-well plate or tubes

Homogenize samples with TissueLyser II, TissueLyser LT or vortexer

Centrifuge

Transfer supernatant to fresh 96-well plate or tubes

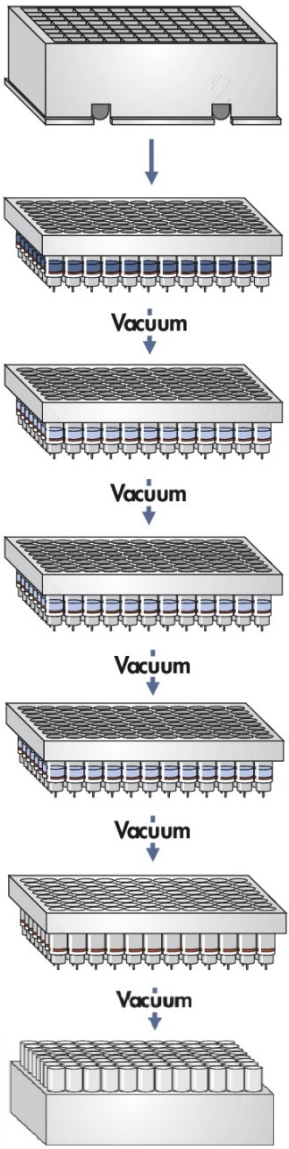
Add Buffer C3. Incubate 5 min at 4° C

Centrifuge

Transfer supernatant to fresh S-Block

Transfer S-Block containing samples to QIAcube HT instrument

QIAcube HT QIAamp 96 PowerFecal 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.

For all preparations:

- Pipets and disposable pipet tips with aerosol barriers (20–1000 µl)
- Ethanol (96–100%)*
- QIAcube HT Instrument
- QIAcube HT Prep Manager Software
- QIAcube HT Reagent troughs
- Vortexer or TissueLyser II (cat. no. 85300)
- Spatula (e.g., Sarstedt cat. no. 81.970)†
- Ice

For preparation in 2 ml tubes:

- Microcentrifuge with rotor for 2 ml tubes
- Pathogen Lysis Tubes L (cat. no. 19092)

For preparation in 96-well format:

- Centrifuge with rotor for 96-well microplates
- Adapter Bottom Plates (2) (cat. no. 69983)
- PowerBead DNA Plates, Garnet (cat. no. 12955-4-BP)
- S-Blocks (cat. no. 19585)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

### Using stool tubes

For easier measurement of stool samples, stool tubes with integrated measuring spoons in their lids are available, e.g., Sarstedt cat. no. 80.734 or Böttger cat. nos. 07.023.2007 and 07.033.2007†

### TissueLyser II

The TissueLyser II provides high-throughput processing for simultaneous, rapid, and effective disruption of up to 192 biological samples, including all types of human and animal tissue. Processing of up to 2 x 96 samples takes as little as 2–5 minutes. Disruption and homogenization using the TissueLyser II gives yields comparable or better than with traditional rotor–stator homogenization methods. With rotor–stator homogenization, the samples must be processed individually, and the rotor–stator homogenizer must be cleaned after each sample to prevent cross-contamination. In contrast, the TissueLyser II provides simultaneous disruption for high-throughput processing of a variety of human and animal tissues.

The TissueLyser II includes a number of different accessories for ease of use with different sample sizes and throughputs. For simultaneous disruption of up to 48 samples in single tubes, we recommend using Pathogen Lysis Tubes (cat. no. 19092) in combination with the TissueLyser Adapter Set 2 x 24 (cat. no. 69982). For processing samples in 96-well format, we recommend using the PowerBead DNA Plate, Garnet (cat. no. 12955-4-BP). Please note that the PowerBead DNA Plate does not fit into the regular TissueLyser 96-well top plate, so two bottom plates must be used. You can use two bottom plates from the TissueLyser Adapter Set 2 x 24 or 2 x 96, or you can order a set of two bottom plates separately: TissueLyser Adapter Bottom Plates (cat. no. 69983). See “Ordering Information”, page 32.

# Important Notes

## RNase digestion

As an optional step, it is possible to remove RNA from the purified samples via RNase digestion. If RNA-free genomic DNA is required, add 4 µl RNase (100 mg/ml) (cat. no. 19101) to each sample before processing on the QIAcube HT.

## Processing fewer than 96 samples per run

If processing fewer than 96 samples, reuse of unused portions of QIAamp 96 plates, S-Block and EMTR is possible up to three times.
**Note**: We recommend using fresh plasticware for every run. If reusing, take extreme care to prevent cross-contamination.

- Store plates in a way that separates the outlet nozzles under the plate, for example, in the S-Block used in the same run or in a fresh 96-well microtiter plate.
- Cover unused wells of the S-Block and QIAamp 96 plate with a tape sheet at all times.

## Preparing reagents

### QIAGEN Proteinase K

The QIAamp 96 PowerFecal QIAcube HT Kit contains ready-to-use QIAGEN Proteinase K supplied in a specially formulated storage buffer. The activity of the QIAGEN Proteinase K solution is 600 mAU/ml.

QIAGEN Proteinase K is stable for at least 1 year after delivery when stored at room temperature (15–25°C). To store for more than 1 year or if ambient temperature often exceeds 25°C, we recommend storing QIAGEN Proteinase K at 2–8°C.

### Buffer PW1

Warm Buffer PW1 at 60°C for 10 minutes before use. The solution should still be warm when used. Make sure that there are no precipitates remaining in the solution.

### Buffer C4

Please shake buffer C4 before use.

### Buffer AW1

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

### Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time add ethanol (96–100%) as indicated on the bottle to obtain a working solution. Tick the check box on the bottle label 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 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.

TopElute Fluid might be found as a top layer over the elution buffer. 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 1 illustrates the assembly of the vacuum chamber. For further information, please refer to the *QIAcube HT User Manual*.

1. Insert the channeling block holder into the left (waste) chamber of the vacuum chamber.
2. 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.
3. Then, place the channeling block into the channeling block holder.
4. 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.
5. 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 the top right position.
6. Load an elution microtubes rack (EMTR) into the elution chamber.

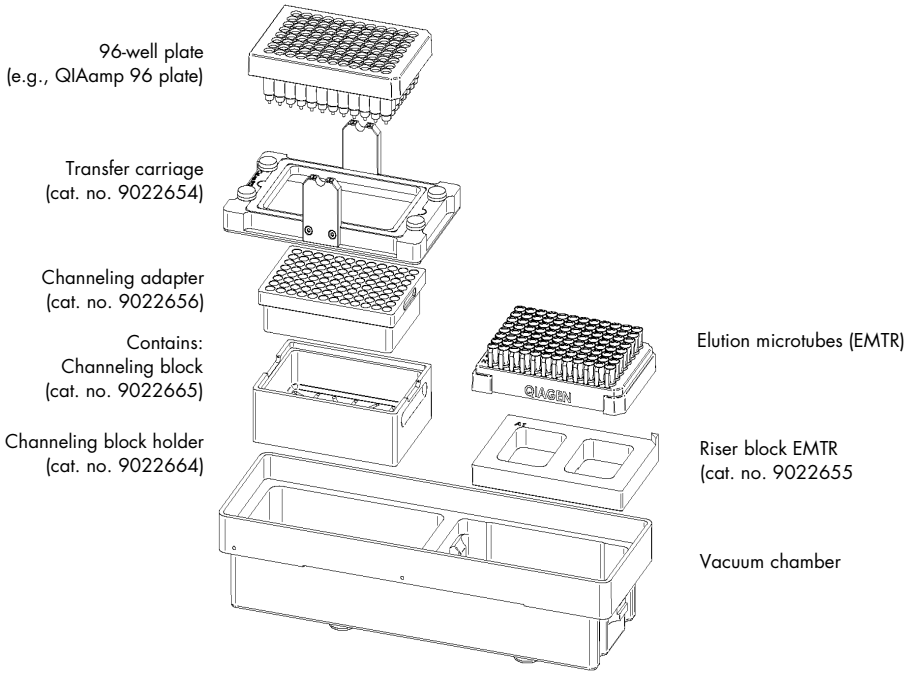



**Figure 1. Assembling the vacuum chamber.**

# QIAamp PowerFecal Protocols

These protocols are for purification of total DNA from up to 96 fresh or frozen stool samples. If you use dried or dehydrated stool samples, we recommend that you resuspend the sample in Buffer PM1 (cat. no. 2600-50-1).

## Important points before starting

- Read "Important Notes" on page 13.
- Do not overload the QIAamp membrane as this can lead to impaired nucleic acid extraction and/or performance in downstream assays.
- Avoid repeating freezing and thawing of samples as this may reduce nucleic acid yield and quality.
- For lysis in a 96-well format using the TissueLyser II, you will also need the following: PowerBead DNA Plates, Garnet (cat. no. 12955-4-BP) and Adapter Bottom Plates (cat. no. 69983).
- For lysis in individual 2 ml tubes, we recommend Pathogen Lysis Tube L (cat no. 19092).

## Things to do before starting

- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).
- Check for precipitates in reagents. If a reagent contains precipitates, incubate at 37°C with gentle shaking to dissolve precipitates. Avoid vigorous shaking, which causes foaming.
- Warm Buffer PW1 at 60°C for 10 minutes before use. The solution should still be warm when used. Make sure that there are no precipitates remaining in the solution.
- Check that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on the bottles to obtain working solutions.

- Ensure that the relevant version of the **QIAamp 96 PowerFecal QIAcube HT** protocol is available on the computer.

  QIAcube HT Prep Manager protocol files (file extension ***.qpmx**), which contain all the information required to perform a run on the QIAcube HT instrument, are available from **www.qiagen.com/p/QIAcubeHT**, under the **Resources** tab.
- Ensure that QIAcube HT Prep Manager Software is installed.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

## Preparing the samples

1. Place up to 200 mg of each stool sample into a Pathogen Lysis Tube L (cat. no.19092) or a PowerBead DNA Plate, Garnet (cat. no. 12955-4-BP). If using tubes, close each tube and place it on ice.

   **Note**: If using stool tubes with integrated spoons in the caps, 200 mg corresponds to approximately one level spoonful of sample.
2. Add 650 µl prewarmed Buffer PW1 to each sample. After addition of Buffer PW1, all of the following steps can be carried out at room temperature (15–25°C).
3. Homogenize each sample thoroughly by one of these methods:
   - Vortex continuously for 10 min or until the stool sample is thoroughly homogenized.
   - Homogenize the samples using the TissueLyser II for 5 min at 30 Hz. If using the PowerBead DNA Plate, Garnet, please use two bottom plate adapters for disruption in the TissueLyser. For more information see page 12.

   **Note**: Vortexing is recommended when isolating human DNA. Homogenization using the TissueLyser II is recommended when isolating pathogen DNA to ensure complete disruption of microorganisms.

   **Note**: It is important to homogenize the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.
4. Centrifuge at full speed for 1 min (tubes) or 5 min (plate) to pellet stool particles.

5. Pipet approximately 400 µl supernatant from step 4 into a new tube or S-Block.

   **IMPORTANT**: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again

   **Note**: For certain types of stool samples (e.g., dehydrated stool), it might be necessary to use more Buffer PW1 to obtain 400 µl of supernatant.
6. Add 150 µl Buffer C3 to the supernatants and mix thoroughly by carefully vortexing or pipetting.
7. Incubate for 5 min at 4 °C.
8. Centrifuge at full speed for 1 min (tubes) or 5 min (S-Block).
9. For each sample, add 20 µl Proteinase K to a new S-Block well. Add 300 µl of each supernatant from step 8 to these wells and mix. Incubate for 10 min at room temperature (15–25°C).

   **Note**: If you only want to isolate host DNA from the stool samples, leave out the Proteinase K and incubation step, and only transfer 300 µl of supernatant to the S-Block.

   **Note**: If RNA-free genomic DNA is required, add 4 µl RNase (100 mg/ml) (cat. no. 19101) to each sample before processing on the QIAcube HT.
10. Proceed with "QIAamp DNA Protocol on the QIAcube HT".

## QIAamp DNA Protocol on the QIAcube HT

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 **QIAamp 96 PowerFecal** experiment.
4. Enter the kit information.
5. Select the pre-treatment from the drop-down menu.

   **Note**: The selected sample type will determine which pre-treatment options appear.

**Note**: The sample type and pre-treatment information are only for documentation and do not influence the purification protocol.

6. Select the protocol: QIAamp PowerFecal protocol.
7. Optional: Set optional steps for checking vacuum performance.

   **Note**: The run script provided by QIAGEN includes all necessary steps to guarantee best performance. Nevertheless, there is the possibility to check the vacuum performance during the run. This option is selected by default in the QIAamp PowerFecal protocol. Therefore, the instrument will pause after the binding step, so you can check if any wells may be clogged. For more information, see section "Vacuum performance check" on page 24.
8. Click **Next**.
9. Optionally, click **Save**. With QIAcube HT Prep Manager software it is possible to save an experiment at any step in the run setup procedure.
10. In the **Labware Selection** screen, select **Use sample ID or existing sample input file** and define the samples.

    To enter sample IDs manually or to automatically generate sample IDs, select **Enter sample IDs**. Then, start naming the samples by clicking **Define Samples**. For information about how to automatically create sample IDs, refer to the *QIAcube HT User Manual*. Alternatively, to load an existing sample file select **Load existing sample files** and then either click **Load sample file** or use the **Scan** button to import the data.

    **Note**: Changing the input labware is not recommended by QIAGEN.
11. In the **Plate Assignment** screen, select the samples from the input and assign them to the output plate. Then, click **Next**.

    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.

12. In **Worktable setup** screen, click on any worktable positions marked with yellow color and follow the instructions to set up the 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*.

13. Confirm that the liquid waste level in the waste bottle matches the level indicated in the software. The waste bottle status has three states: green indicates that the bottle has enough space for another run; yellow indicates that the bottle is nearly full, but the run can be started; red indicates that the bottle needs to be emptied before starting the run.

14. 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. 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 1 on page 16. This 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 (supplied). Unused wells must be sealed to ensure proper vacuum operation.

    **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 lysate transfer.
    Check that the number and position of available and unused tips is the same on the instrument worktable and in the software workspace.
    If more tips are required, you will be prompted to replace empty tip racks with new tip

racks during the run. Information about the approximate time for refill will be given in the run screen. For more information, see the *QIAcube HT User Manual*.

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 S-Block containing the samples in the indicated position of the QIAcube HT worktable.

    **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 volume.
19. Close the instrument hood and start the run by clicking **Start run**.
20. The protocol run begins.

    **Important**: At the beginning of each run, an open circuit test and a plate detection test are performed automatically. If the QIAamp 96 plate in the transfer carriage is improperly aligned to the left compartment of the vacuum chamber, you will be prompted to position it correctly. After adjusting the position, click **Retry** to initiate the tests again.

    For more information about the system tests, see the *QIAcube HT User Manual*.

    **Note**: The vacuum performance check is activated. Therefore, the protocol will stop for a manual interaction after the binding step.
21. After the protocol is finished, create a report if necessary.
22. Cover the elution plate (EMTR) with the lid and remove from the elution chamber.
23. Two liquid phases might be found in the Elution Microtubes. If this is the case, TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

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

## Cleaning the instrument after completing a run

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 the lid and remove them from the worktable.
3. Cover fractions of partly used tip racks with an adhesive tape. Then cover the tip racks with the lid and remove from the worktable. Discard empty tip racks.
4. If the run has been stopped and the instrument did not remove all used tips, remove them now and discard them.
5. Remove all reagent troughs and discard them.

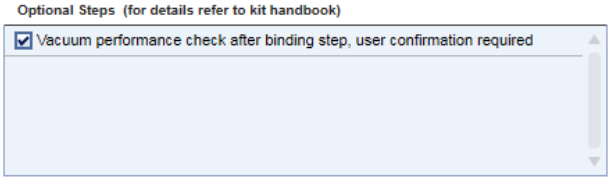
   **Note**: We recommend not reusing reagents for multiple runs.
6. Remove the input plate.
7. Discard the QIAamp 96 plate or keep partially used QIAamp 96 plates for subsequent reuse. In this case cover used fractions with an adhesive tape.
8. Remove the tip chute and all adapters from the worktable. 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. Clean any reagents that may have spilled on the instrument worktable or vacuum chamber with a damp cloth.
11. Discard all waste according to local safety regulations.

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

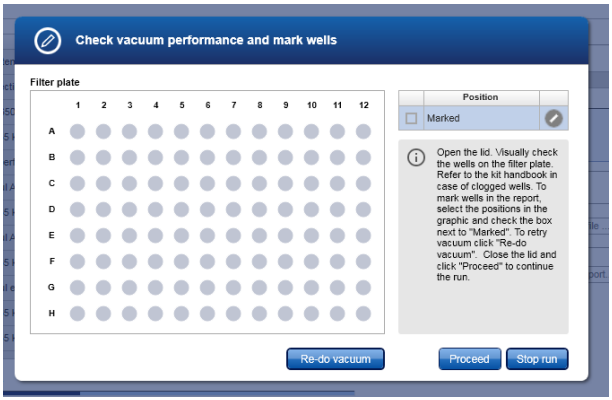
## Optional steps

### Vacuum performance check

Using the vacuum performance check option results in one manual interaction pause after the binding step. This optional setting allows the user to check whether all the liquid has passed through the membranes. By default, this step is checked.




If the vacuum performance check step is checked, the instrument will pause after the binding step. The user can then look to see whether all liquid has passed through the membranes and decide whether to switch on the vacuum again (**Re-do vacuum**) or to continue (**Proceed**).




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.

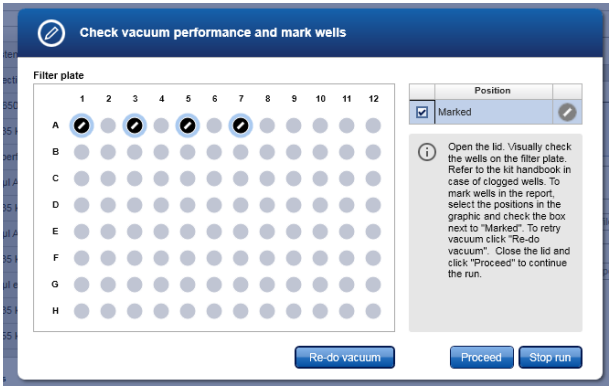
   If liquid remains in the wells, click the **Re-do vacuum** button to apply the same vacuum pressure again. The vacuum will be activated for a certain time or until you press the **Stop vacuum** button.

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.

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

   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**.



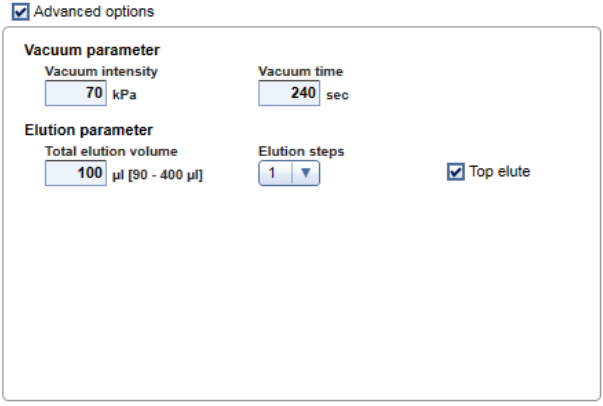

4. If liquid still remains in any well, manually remove the liquid using a pipet.
5. After the instrument has added additional reagents, open the hood to pause the run. Check to see whether the affected well is still blocked. If so, manually remove the liquid from the affected well using a pipet.
6. Either click **Proceed** to continue the run, or click **Stop run** to stop the run.

## 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. QIAGEN is not responsible for the outcome and does not support experiments performed using modified advanced options. 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.




### Vacuum parameter

In the **Vacuum parameter** section, it is possible to change two parameters: vacuum intensity and vacuum time.

The default settings are 70 kPa for the vacuum intensity and 240 sec for the vacuum time.

If you want to adapt your protocol you could change the vacuum intensity from 35 kPa to 70 kPa. Please note that these changes are not recommended by QIAGEN.

If you change the vacuum intensity parameter, this only affects the vacuum intensity following the binding step. All other vacuum steps will be unaffected.

If you want to adapt your protocol you could change the vacuum time from 60 sec to 480 sec. Please note that these changes are not recommended by QIAGEN.

If you change the vacuum time parameter, this will only affect the 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 and the elution step.

The recommended values for these parameters are shown in the QIAcube HT Prep Manager Software.

If you want to adapt your protocol you could change the total elution volume to another value within the defined range. Please note that these changes are not recommended by QIAGEN.

### TopElute

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.

In case TopElute Fluid should not be used during the run, uncheck the **TopElute** option under **Advanced** options.

**Important**: Changing the usage of TopElute Fluid is not recommended or tested by QIAGEN.

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

**Note**: TopElute Fluid might be found as a top layer over the elution buffer. It is inert and has no effects on downstream applications.

# 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** |
|---|---|
| **Instrument issues** | |
| a) Recovery in case of user interruption | The QIAcube HT interrupts a run upon opening of the hood. 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 potentially block the membrane, we recommend using a vacuum performance check.<br>If liquid is still visible, remove all liquid using a pipet. Then scrape the surface of the membrane with a fresh pipet tip in order 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.<br>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 pipet.<br>Do not perforate the membrane. Uncovered perforated wells will disturb vacuum integrity during elution across the whole plate.<br>Proceed with the run.<br>Next time, use less sample. |
| c) Instrument failure/cancelled run | It is not possible to recover the run. Please continue manually. |
| **Little or no DNA in the eluate** | |
| a) Sample stored incorrectly | Samples should be stored at 2 to 8°C or –20°C. |
| b) Buffer PW1 not prewarmed | Using Buffer PW1 that has not been prewarmed can lead to incomplete sample lysis. |

| | Comments and suggestions |
|---|---|
| c) Insufficient sample lysis | QIAGEN Proteinase K was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh QIAGEN Proteinase K (see "Storage" on page 5).<br>Repeat the DNA purification procedure with a new sample. Be sure to mix the sample until the sample is thoroughly homogenized. |
| **$A_{260}/A_{280}$ ratio of purified nucleic acids is too low** | |
| a) Buffer AW1 or Buffer AW2 prepared incorrectly | Check that Buffer AW1 or Buffer AW2 concentrate was diluted with the correct volume of ethanol, as indicated on the bottle. Use 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification protocol with new samples. |
| b) Buffer AW1 or Buffer AW2 prepared with 70% ethanol | Check that Buffer AW1 and Buffer AW2 concentrates were diluted with 96-100% ethanol. Repeat the purification procedure with a new sample. |
| **DNA does not perform well in downstream applications** | |
| a) Little or no DNA in the eluate | See "Little or no DNA in the eluate", above. |
| b) Reduced performance in PCR | Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly. |
| c) Performance of purified nucleic acids in downstream assays varies according to their original positions on the QIAamp 96 plate | Salt and ethanol components of Buffer AW1 or Buffer AW2 may have separated out after being left for a long period between preparations. Always mix buffers thoroughly before each preparation. |
| d) Elution microtubes autoclaved before elution | Do not autoclave elution microtubes. Autoclaving may leach chemicals that can inhibit some downstream enzymatic reactions from the walls of elution microtubes. Repeat the procedure with a new set of elution microtubes. Elution microtubes are delivered RNase and DNase-free. |
| **Precipitate in buffers** | |
| a) Precipitate in Buffer PW1 | Precipitate may form after storage at room temperature. To dissolve precipitate, incubate Buffer PW1 for several min at 60°C with occasional shaking. |

# Appendix A: Determination of Concentration, Yield, Purity and Length of DNA

## Determination of concentration, yield and purity

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an $A_{260}/A_{280}$ ratio of 1.7–1.9.

Absorbance readings at 260 nm should lie between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer. To measure only DNA, a fluorometer must be used.

## Determination of DNA length

The length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol precipitation and reconstituted by gentle agitation in approximately 30 µl TE buffer, pH 8.0, for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature (15–25°C) since over-dried genomic DNA is very difficult to redissolve.

Load 3–5 µg DNA per well. Standard PFGE conditions are as follows:

- 1% agarose gel in 0.5x TBE electrophoresis buffer
- Switch intervals: 5–40 s
- Run time: 17 h
- Voltage: 170 V

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAamp 96 PowerFecal QIAcube HT Kit | For 480 preps: QIAamp plates, QIAGEN Proteinase K, Buffers | 51531 |
| QIAcube HT Plasticware | For 480 preps: 5 S-Blocks, 5 EMTR RS, 2 x 50 Caps for EMTR, 9 x 96 Filter-Tips OnCor C, TapePad | 950067 |
| Elution Microtubes RS | 24 x 96 Elution Microtubes, racks of 96; includes cap strips | 120008 |
| S-Blocks | 24 x 96-well blocks with 2.2 ml wells | 19585 |
| Buffer PM1 | 36 ml Buffer PM1 | 2600-501 |
| **TissueLyser** | | |
| TissueLyser II | Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96* | 85300 |
| TissueLyser Adapter Set 2 x 24 | 2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II | 69982 |
| TissueLyser Adapter Set 2 x 96 | 2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II | 69984 |
| TissueLyser LT | Compact bead mill, 100-240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube† | 85600 |

* The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96.

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

| Product | Contents | Cat. no. |
|---|---|---|
| TissueLyser LT Adapter, 12-Tube | Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT | 69980 |
| Stainless Steel Beads, 5 mm (200) | 200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems | 69989 |
| Adapter Bottom Plates (2) | 2 Adapter Bottom Plates for processing PowerBead DNA Plates on the TissueLyser II | 69983 |
| PowerBead DNA Plates, Garnet (4) | 96-well blocks containing Garnet beads and silica mat | 12955-4-BP |
| **QIAcube HT instrument** | | |
| QIAcube HT System | Robotic workstation with UV lamp, HEPA filter, laptop, QIAcube HT operating software, start-up pack, installation and training, 1-year warranty on parts and labor | 9001793 |
| **Accessories** | | |
| Collection Microtubes (racked) | (10 x 96) nonsterile polypropylene tubes; 960 in racks of 96 | 19560 |
| Collection Microtube Caps | For capping collection microtubes and round-well blocks; 960 in strips of 8 | 19566 |

For a complete list of accessories, visit **www.qiagen.com/p/QIAcubeHT**.

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.

# Quick-Start Protocol

This protocol is for use with QIAcube HT Prep Manager software.

## Further information

- *QIAamp 96 PowerFecal QIAcube HT Handbook:* **www.qiagen.com/HB-2162**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- This protocol is for the purification of nucleic acids from stool samples using the QIAamp 96 PowerFecal QIAcube HT Kit with the QIAcube HT Prep Manager software.
- Do not overload the QIAamp membrane as this can impair nucleic acid extraction and/or performance in downstream assays. See the kit handbook for more information.
- Avoid repeated freezing and thawing of samples, as this may reduce nucleic acid yield and quality.
- Prepare Buffers AW1 and AW2 according to the instructions in the kit handbook.
- Warm Buffer PW1 at 60° C for 10 min before use. The solution should still be warm when used. Make sure there are no precipitates in the solution.

## Preparing the samples

1. Place 200 mg of each stool sample into a Pathogen Lysis Tube L (cat. no.19092) or a PowerBead DNA Plate, Garnet (cat. no. 12955-4-BP). If using tubes, close each tube and place it on ice.

2. Add 650 µl prewarmed Buffer PW1 to each sample. After addition of Buffer PW1, all of the following steps can be carried out at room temperature (15–25°C).

   **Note**: If working with dehydrated stool samples, it might be necessary to increase this volume. Resuspend the stool sample in enough Buffer PW1 (cat. no. 2600-50-1) to obtain 400 µl of supernatant after centrifugation in step 4.

3. Homogenize each sample thoroughly by one of these methods:
   - Vortex continuously for 10 min or until the stool sample is thoroughly homogenized.
   - Homogenize the samples using the TissueLyser II for 5 min at 30 Hz. If using the PowerBead DNA Plate, Garnet and the TissueLyser II for disruption, please also use two TissueLyser Adapter Bottom Plates (cat. no. 69983). For more information see the kit handbook.

   **Note**: Vortexing is recommended when isolating human DNA. Homogenization using the TissueLyser II is recommended when isolating pathogen DNA to ensure complete disruption of microorganisms.

   **Note**: It is important to homogenize the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

4. Centrifuge at full speed for 1 min (tubes) or 5 min (plate) to pellet stool particles.
5. Pipet approximately 400 µl supernatant from step 4 into a new tube or S-Block (cat. no. 19585) (not provided).

   **IMPORTANT**: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again

6. Add 150 µl of Buffer C3 to each supernatant and mix thoroughly by carefully vortexing or pipetting.
7. Incubate for 5 min at 4°C.
8. Centrifuge at full speed for 1 min (tubes) or 5 min (S-Block).
9. For each sample, add 20 µl Proteinase K to a new S-Block well. Add 300 µl of each supernatant from step 8 to these wells and mix. Incubate for 10 min at room temperature (15–25°C).

**Note**: If you only want to isolate human DNA from the stool samples, leave out the Proteinase K and incubation step, and only transfer 300 µl of supernatant to the S-Block.

## QIAamp 96 PowerFecal Protocol on the QIAcube HT

1. Start the QIAcube HT Prep Manager software. Click on the **Home** icon in the main toolbar to access the Home screen.
10. Select QIAamp 96 PowerFecal from the **Create Experiment** list. Follow the instructions in the wizard and fill in all required fields.
11. In the **Setup** step, select **Sample type** and **Pre-treatment** for documentation.
12. Select the protocol: QIAamp 96 PowerFecal protocol. For information about optional steps and advanced options see the kit handbook.
13. Define samples in the **Labware selection** step.
14. Arrange samples to the output plate in the **Assignment** step. **Note**: The instrument must be switched on and connected to the software before entering the **Worktable** step.
15. Follow the instructions for loading the worktable.
16. Add the volume of sample indicated in the **Worktable** step to the selected S-Block wells.
17. Save the experiment by clicking the **Save** button in the button bar.
18. Click the **Start** run button to start the run.
    **Important**: The protocol includes a vacuum performance check after the first vacuum step. Therefore, the software will show a dialog that needs to be confirmed after the vacuum has finished.
19. When the protocol is complete, cover the elution plate (EMTR) with the lid and remove it from the elution chamber.
    **Note**: If using Top Elute fluid, there may be 2 liquid phases in the elution microtubes. Top Elute fluid will be the top layer over the elution buffer.
20. Create a report (if required).
21. Follow the cleaning procedure.

**Limited License Agreement for QIAamp 96 PowerFecal QIAcube HT Kit**

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

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