November 2022

QIAamp® 96 DNA QIAcube® HT Handbook

For automated purification of total DNA from blood and tissue using QIAcube HT and QIAcube HT Prep Manager Software

Sample to Insight

Contents

Kit Contents

QIAamp 96 DNA QIAcube HT Kit	(5)
Catalog no.	51331
Number of preps	480
QIAamp 96 plates	5
Buffer ATL	2 x 50 mL
Buffer VXL*	2 x 30 mL
Buffer ACB*† (concentrate)	2 x 60 mL
QIAGEN® Proteinase K	2 x 6 mL
Buffer AW1*‡ (concentrate)	2 x 98 mL
Buffer AW2‡§ (concentrate)	2 x 66 mL
Buffer AE	2 x 110 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 isopropanol as indicated on the bottle to obtain a working solution.
‡ Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.
§ Contains sodium azide as a preservative.

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 and Elution Microtubes RS (including caps for strips). See “Ordering Information,” page 35.

Shipping and Storage

QIAamp 96 plates and all buffers are stable for 1 year at room temperature (15–25°C) and dry conditions 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 DNA QIAcube HT Kit is intended for the automated extraction of DNA using the QIAcube HT instrument. The QIAamp 96 DNA 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 	CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffer VXL and Buffer AW1 contain guanidine hydrochloride, and Buffer ACB contains guanidine thiocyanate, both of 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 DNA QIAcube HT Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAamp 96 DNA QIAcube HT Kit uses well-established technology for purification of total DNA (e.g., genomic, mitochondrial, and pathogen) from a variety of sample sources including fresh or frozen tissues and cells, blood or bacteria. The procedure yields high-quality DNA that performs well in PCR and other enzymatic reactions.

The QIAamp 96 DNA 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 µL in volume, on the QIAcube HT instrument system (in increments of 8). The virtual worktable in the QIAcube HT Prep Manager Software permits easy operation of the QIAcube HT instrument by reflecting the actual worktable on the apparatus. Blood and buffy coat samples for processing can be fresh or frozen, provided they have not been frozen and thawed more than once (see "Important Notes," page 11). The procedure can be used for samples treated with citrate, heparin, or EDTA. Purification requires no organic extraction or alcohol precipitation. DNA is eluted in Buffer AE, and is free of proteins, nucleases, and other contaminants or inhibitors. It is ready for use in enzymatic reactions, such as PCR, or storage at –15°C to –30°C. DNA purified using the QIAamp 96 DNA QIAcube HT Kit is up to 50 kb in size, with fragments of approximately 20–30 kb predominating. DNA of this length denatures completely during thermal cycling and can be amplified very efficiently.

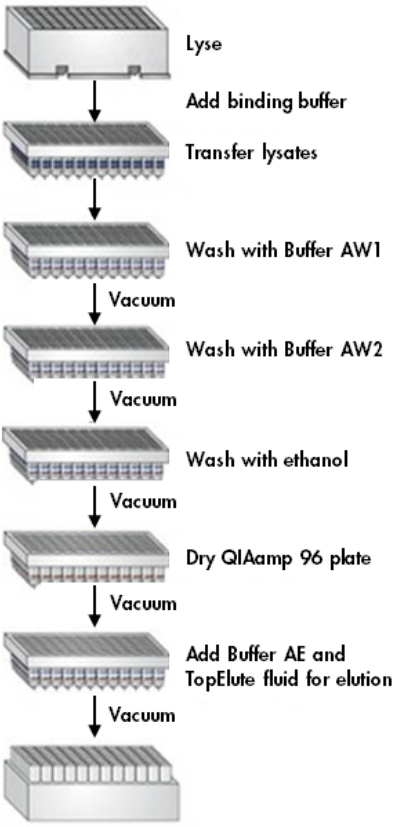
The fully automated procedure requires <2 hours to process 96 samples, with no hands-on time.

Principle and procedure

The QIAamp 96 DNA QIAcube HT procedure is simple (see flowchart on the next page). Samples are first lysed using QIAGEN Proteinase K. 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 as contaminants pass

through. Remaining contaminants and enzyme inhibitors are removed in three efficient wash steps. Pure DNA is eluted under vacuum in a single step in approximately 200 µL of Buffer AE equilibrated to room temperature (15–25°C). DNA recovery is enhanced by overlaying the elution buffer with TopElute Fluid. QIAamp purified DNA has A_{260}/A_{280} ratios of 1.7–1.9, and absorbance scans show a symmetric peak at 260 nm, confirming high purity.

QIAamp 96 DNA QIAcube HT 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.

For all protocols:

- Pipettes and disposable pipette tips with aerosol barriers (20–1000 µL)
- Isopropanol (100%)
- Ethanol (96–100%)*
- Phosphate-buffered saline (PBS), pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)
- QIAcube HT Instrument
- QIAcube HT Prep Manager Software
- QIAcube HT Reagent Troughs†
- Vortexer

For purification from tissue samples

- Collection Microtube Racks†
- Collection Microtube Caps†
- Tissue disruption system such as the TissueLyser II†
- 5 mm stainless steel beads†
- Centrifuge

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
† For ordering, see "Ordering Information" on page 34.

TissueLyser II

The TissueLyser II includes a number of different accessories for ease of use with various sample sizes and throughputs. The TissueLyser Adapter Set 2 x 96 allows simultaneous processing of up to 192 samples in collection microtubes. Stainless steel beads with a diameter of 5 mm provide optimal disruption of human and animal tissue. The TissueLyser 5 mm Bead Dispenser, 96-Well, is also available to conveniently deliver 96 beads in parallel into collection microtubes. See "Ordering Information," page 34.

Important Notes

Starting materials

Blood

Whole blood samples treated with EDTA, citrate, or heparin can be used, and may be fresh or frozen. Yield and quality of the purified DNA depend on storage conditions of the blood. Fresher blood samples yield better results. For long-term storage, we recommend collecting blood samples in tubes containing anticoagulant and storing at –65°C to –90°C.

Repeatedly frozen and thawed samples, samples stored at room temperature (15–25°C) for extended periods, or samples containing visible precipitates should not be processed. Processing of samples containing visible precipitates can lead to reduced DNA size. Use of poor-quality starting material will also lead to reduced length and yield of purified DNA.

Tissue and cells

To obtain optimum DNA yield and quality, it is important not to overload the QIAamp 96 plate, as this can lead to significantly lower yields than expected. For samples with very high DNA content (e.g., spleen, which has a high cell density, and cell lines with a high degree of ploidy, as well as lung and liver tissue), less than the recommended amount of 20 mg should be used. If you have no information regarding DNA content, we recommend beginning with less than 10 mg of starting material. Depending on the yield obtained, the sample size can be increased in subsequent preparations. After proteinase K digestion, tissue samples can also be stored in Buffer ATL for up 6 months at ambient temperature without any reduction in DNA quality.

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) to each sample.

> **Note**: Generally, the QIAamp 96 DNA QIAcube HT Kit co-purifies DNA and RNA. For certain DNA applications that might be sensitive to presence of RNA, removal may be desirable.

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 DNA 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.

For processing blood and cells, add proteinase K to Buffer VXL immediately before starting the protocol.

For processing human and animal tissue, the proteinase K digestion is carried out in ATL buffer prior to the purification protocol.

Buffer ACB

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

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.

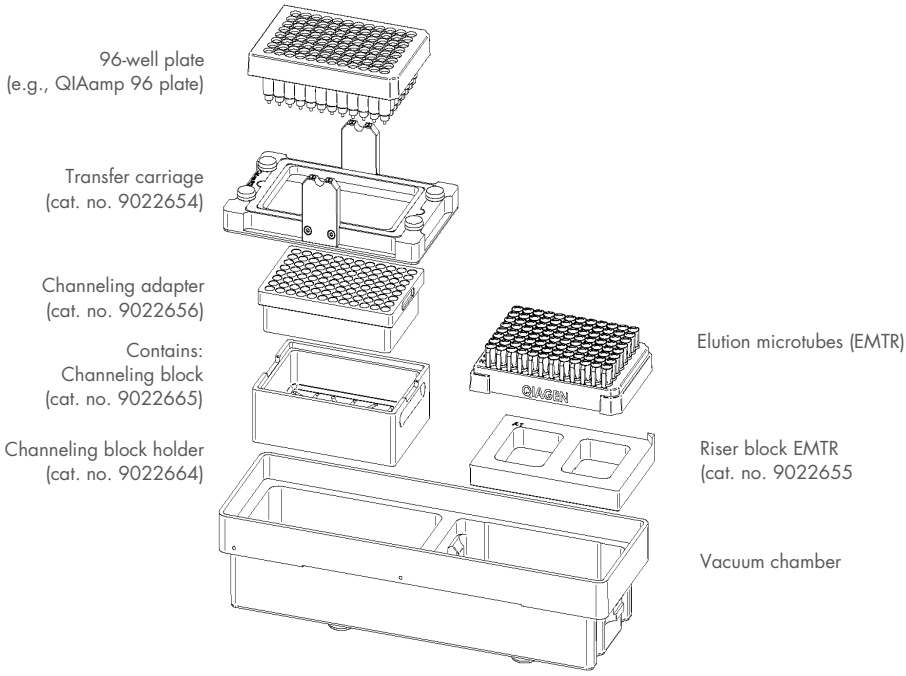


Figure 1. Assembling the vacuum chamber.

QIAamp DNA Protocol

This protocol is for purification of genomic DNA from up to 96 samples from starting materials including blood, cells (maximum of 5 x 10^6), and soft tissues (maximum of 20 mg) such as biopsies, mouse tails, brain, liver, and muscle.

Starting material can be fresh or frozen whole blood, buffy coat, or cell samples. If you want to use frozen tissue samples (maximum of 20 mg) such as biopsies, mouse trails, brain, liver, and muscle, be sure that you carry out sample pre-treatment before starting the purification protocol.

Important points before starting

- Read "Important Notes" on page 11.
- 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 tissue samples, you will also need the following: Collection Microtube Racks (cat. no. 19560) and Collection Microtube Caps (cat. no. 19566).

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.
- Check that Buffer ACB, Buffer AW1, and Buffer AW2 have been prepared according to the instructions in "Preparing reagents" (page 12).

- When working with difficult samples, use a vacuum performance check to verify if all liquid has passed through the membrane.
- Ensure that the relevant version of the **QIAamp 96 DNA 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.

Preparation of blood and cell samples

1. Add 200 µL of each sample to the selected S-Block wells.

   Note: If the volume of the samples is less than 200 µL, add PBS or 0.9% NaCl to a final volume of 200 µL.

   Note: If working with cultured cells, centrifuge the appropriate number of cells (maximum of 5 x 10^6) for 5 min at 300 x *g*. Resuspend each pellet in 200 µL PBS.

   Optional: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL) to each sample and incubate for 2 min at room temperature.
2. Proceed with "QIAamp DNA Protocol on the QIAcube HT" on page 20.

Preparation of tissue samples

Tissue disruption can be performed manually or with a tissue homogenizer such as the TissueLyser instrument. Tissue disruption using the TissueLyser facilitates the digestion process and shortens the time required for digestion (see "Option 2: Sample disruption and homogenization using the TissueLyser II" on page 18).

Option 1: Manual treatment

1. Cut up to 20 mg tissue into small pieces. For rodent tails, place one (rat) or two (mouse) 0.4–0.6 cm lengths of tail into a collection microtube. Earmark the animal appropriately.

   Note: The maximum amount of starting material that can be processed is 20 mg. For some tissues (spleen, lung, and liver), using more than 10 mg may lead to incomplete lysate transfer due to highly viscous lysates.
2. Prepare a proteinase K – Buffer ATL working solution containing 20 µL proteinase K stock solution and 180 µL Buffer ATL per sample, and mix by vortexing.
3. Immediately pipet 200 µL of the proteinase K – Buffer ATL solution into each well containing the cell or tissue samples. Seal the microtubes properly with the collection microtube caps.
4. Place a clear cover of collection microtubes over each rack, and mix by inverting the rack of collection microtubes.
5. Briefly centrifuge the collection microtubes to collect any solution from the caps. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge. Proceed to “Lysis of tissue” on page 19.

   Important: It is essential that the samples are completely submerged in the proteinase K – Buffer ATL working solution after centrifugation.

Option 2: Sample disruption and homogenization using the TissueLyser II

We recommend using the TissueLyser II for disruption and homogenization of up 192 samples in parallel. This protocol describes DNA purification from flash-frozen tissue using the TissueLyser II.

1. Place 5 mm stainless steel beads into the collection microtubes (1 bead per tube).
2. Determine the amount of tissue per sample by weighing the samples. Do not use more than 20 mg tissue cut into small pieces. Transfer tissue to a collection microtube. For

rodent tails, place one (rat) or two (mouse) 0.4–0.6 cm lengths of tail into a collection microtube. Earmark the animal appropriately.

Important: The maximum amount of starting material that can be processed is 20 mg. For some tissues (spleen, lung, and liver), using more than 10 mg may lead to incomplete lysate transfer due to highly viscous lysates.

3. Cover the tissue with 180 µL Buffer ATL and seal the microtubes properly with collection microtube caps.
4. Sandwich each rack of collection microtubes between TissueLyser Adapter plates and fix into TissueLyser clamps as described in the *TissueLyser User Manual*. Operate the TissueLyser II for 20 s at 15 Hz.

   Note: Exceeding this time and intensity may lead to significant fragmentation of genomic DNA.
5. Centrifuge the collection microtubes briefly to collect any solution from the caps.
6. Add 20 µL proteinase K to each sample.
7. Proceed with "Lysis of tissue" below.

Lysis of tissue

1. Incubate samples at 56°C overnight or until the samples are completely lysed. Place a weight on top of the caps during the incubation. Mix occasionally during incubation to disperse the sample, or place on a rocking platform.
2. Ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each rack of collection microtubes and shake the racks vigorously up and down for 15 s.
3. Briefly centrifuge the collection microtubes to collect any solution from the caps. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.
4. Add 200 µL of each lysed sample from the collection microtubes into a fresh S-Block well.

   Optional: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL) to each well and mix carefully by pipetting.

Optional: For RNA digestion, incubate for 5 min at room temperature.

5. Proceed with "QIAamp DNA Protocol on the QIAcube HT" as shown below.

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 DNA** experiment.
4. Enter the kit information.
5. Select the type of sample to be processed. Sample types are combined in categories. Select the category that best fits your sample type.
6. 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.
7. The QIAamp DNA protocol appears.

   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. If this option is selected, 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 25.
8. Click **Next**.
9. Optionally, click **Save**. The QIAcube HT Prep Manager Software allows to save an experiment at any step during 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**. Press and hold the left mouse button to mark the columns and rearrange them as needed.
12. In the **Worktable setup** screen, select any virtual worktable position marked by yellow color and follow the instructions to set up the instrument worktable.

    Important: All positions to be used on the instrument worktable must be calibrated. If the **Calibrate Labware** button appears yellow, calibration is required. For further information, see the *QIAcube HT User Manual*.
13. 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 indicates that no action is necessary; yellow indicates that the bottle is almost full, but the run can be started; red indicates to empty the bottle 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 15. 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 is a sufficient number of tips to follow the protocol at least up to completion of lysate transfer.
    Check that the number and position of available and unused tips on the instrument worktable is the same as 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. Information about the approximate time for refill will be given in the run screen. For more details, see the *QIAcube HT User Manual*.
17. Transfer the indicated volumes of all reagents except the Buffer VXL Mixture into the corresponding reagent troughs. Close the lids and place the troughs in the indicated positions on the worktable.
18. Prepare a mixture of Buffer VXL and proteinase K according to Table 1 on page 23.

    Note: If you use tissues, proteinase K is already used during sample disruption. In this case, load only an appropriate amount of Buffer VXL as indicated on the virtual worktable.

    Important: Prepare the VXL mixture immediately before starting the run.

    For the QIAamp DNA protocol, place the VXL mixture in the indicated positions on the worktable.

Table 1. Preparation of Buffer VXL and proteinase K.

	Number of samples									
	24	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**
Buffer VXL (mL)	2.48	3.2	4.0	4.72	5.44	6.16	6.88	7.68	8.4	9.12
QIAGEN Proteinase K (µL)	620	800	1000	1180	1360	1540	1720	1920	2100	2280

19. Add the samples (as shown on the virtual worktable) to the selected S-Block wells. Place the S-Block 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.

20. Close the instrument hood and start the run by clicking **Start run**.

21. 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*.

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

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

23. Cover the elution plate (EMTR) with the lid and remove from the elution chamber.

24. 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 and discard all reagent troughs.

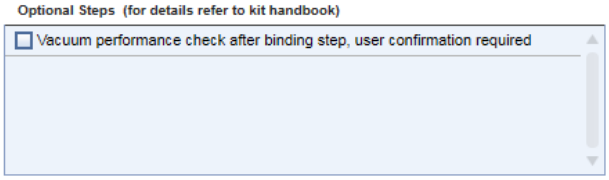
   Note: We recommend not reusing reagents for multiple runs.
6. Remove the input plate.
7. Discard the QIAamp 96 plate or keep partially used QIAamp 96 plates for subsequent reuse. In this case, cover used fractions with an adhesive tape.
8. Remove the tip chute and all adapters from the worktable. Remove 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.

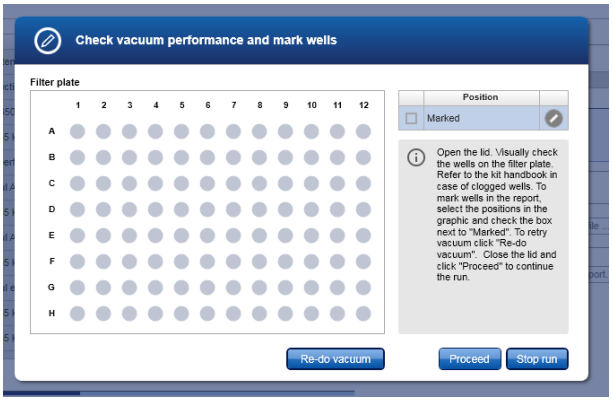
Optional steps

Vacuum performance check

Using the vacuum performance check option results in one manual intermediate step after the binding process. This optional setting allows the user to check whether all the liquid has passed through the membranes. By default, this step is not activated.



If the vacuum performance check step is activated, the instrument will pause after the binding step. If liquid remains on the surface, the user can re-apply vacuum (**Re-do vacuum**); if not, continue with the procedure (**Proceed**).



1. Open the instrument lid.

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

2. Check the wells on the QIAamp 96 plate for any remaining liquid.

3. If no liquid is visible in the wells after the vacuum step, click **Proceed** to continue the run.

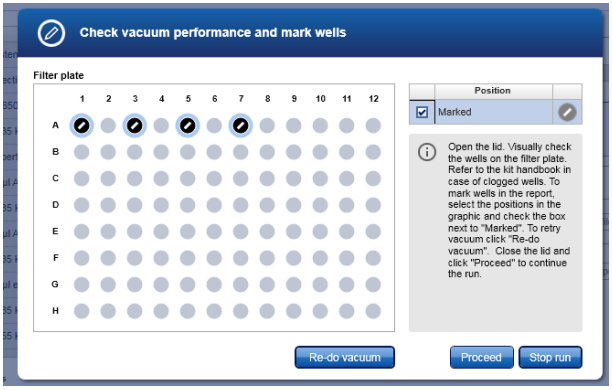
   If liquid remains in the wells, click the **Re-do vacuum** button to apply the same vacuum pressure again. The vacuum will be activated for a certain time or until you press the **Stop vacuum** button.

4. 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.

5. 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 multiple, nonadjacent positions. Alternatively, drag the mouse to select adjacent positions in a rectangle.

6. 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**.



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

8. 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.

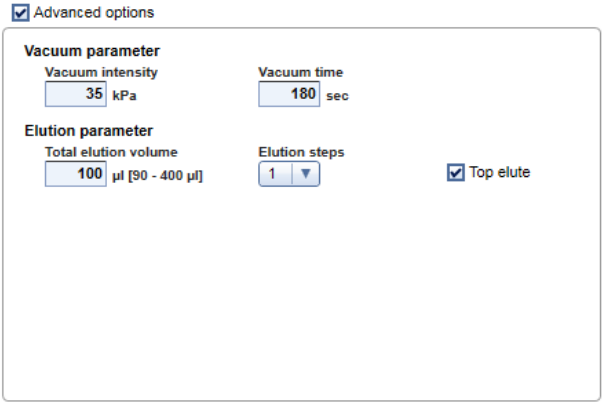
9. Either click **Proceed** to continue the run, or click **Stop run** to stop the run.

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. 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 35 kPa for the vacuum intensity and 300 seconds for the vacuum time.

If you want to adapt your protocol you could change the vacuum intensity from 35 kPa to 70 kPa. 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 seconds to 480 seconds. 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.

In some cases it might be helpful to elute multiple times with a lower volume than one time using the complete elution volume. Increasing the number of elution steps will result in a multiplication of elution buffer distribution, incubation pause, and vacuum step(s) without influencing the total amount of elution volume.

If you want to adapt your protocol you could change the elution step from 1 to 2.

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 TopElute option is checked.

If the TopElute Fluid will 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 in 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 or protocols in this handbook (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
Instrument issues	
a) Recovery in case of instrument failure or 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 enabling the optional vacuum performance check step. a) Sample not well lysed. If liquid is still visible after vacuum, remove the liquid by using a pipette. Then scrape the surface of the membrane with a fresh pipette 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, pipette 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. We do not recommend perforating the membrane. Uncovered perforated wells will disturb vacuum integrity during elution across the whole plate. Next time, use less sample (tissue). b) White blood cell count is too high. Dilute samples at least 1:1 with PBS, mix thoroughly (e.g., by inverting the tubes several times), and repeat the procedure. c) Blood and buffy coat samples were processed together in the same protocol run. Blood samples have lower white blood cell counts compared with buffy coat samples, and pass more quickly through the membranes of the QIAamp 96 plate. This leads to a reduction in the vacuum applied to the QIAamp 96 plate, causing inefficient processing of buffy coat samples through the membranes of the QIAamp 96 plate. Repeat the procedure using only blood samples or only buffy coat samples in the same protocol run.

	Comments and suggestions
c) Instrument failure/canceled run	It is not possible to recover the run. Please continue manually.

Little or no DNA in the eluate

a) Buffer ACB prepared incorrectly	Check that Buffer ACB concentrate was diluted with the correct volume of isopropanol, as indicated on the bottle. Use 100% isopropanol. Repeat the purification protocol with new samples.
b) Samples not equilibrated to room temperature	Using cold samples can lower the lysis temperature, leading to incomplete sample lysis.
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 "Shipping and Storage" on page 5). For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. In this case, use the off-board lysis protocol.
d) Samples left standing for too long at room temperature	Genomic DNA in the samples has been degraded by nucleases. Repeat the purification procedure with new samples. The sample has separated into 2 phases, an upper plasma phase (containing only low amounts of DNA) and a lower, cell-rich phase (containing high levels of DNA). Repeat the purification procedure, thoroughly mix samples right before a run is started.
e) If water was used for elution, the pH of the water was too low	Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Buffer AE for elution.
f) Buffer VXL- QIAGEN Proteinase K mixture mixed insufficiently	Mix well by pipetting with a large pipette.
g) DNA degraded	DNA may have been degraded by DNases in the starting material. Ensure that samples are processed immediately following collection or removal from storage. Ensure that no DNase is introduced into the reagents during the procedure. Use Buffer AE or DNase-free water for elution.

A_{260}/A_{280} ratio of purified nucleic acids is too low

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.

Comments and suggestions	
DNA does not perform well in downstream applications	
a) Little or no DNA or RNA 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) Animal blood used	Hemoglobin can be difficult to remove from the blood of some animal species (e.g., monkey and mouse) and may interfere with downstream applications.
e) 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.
Colored residues remain on the QIAamp 96 plate after washing	
a) Buffer AW1 or AW2 prepared incorrectly	Ensure that the correct amounts of ethanol were added to Buffer AW1 and AW2 concentrates. Repeat the purification procedure with new samples.
b) Buffer AW1 or AW2 prepared with low percentage ethanol	Ensure that 96–100% ethanol was added to Buffer AW1 and AW2 concentrates. Repeat the purification procedure with new samples.
Precipitate in buffers	
a) Precipitate in Buffer VXL or Buffer ACB	Precipitate may form after storage at low temperature or prolonged storage. To dissolve precipitate, incubate Buffer VXL or ACB for 30 min at 37°C with occasional shaking.
b) Precipitate in sample – Buffer VXL mixture	If using sample fluid containing Buffer ATL, for example, after enzymatic digestion of tissue, precipitate may form after addition of Buffer VXL to the sample. The precipitate does not influence subsequent protocol steps and can be dissolved by brief incubation at 56°C.

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 nm 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 seconds
- Run time: 17 hours
- Voltage: 170 V

Ordering Information

Product	Contents	Cat. no.
QIAamp 96 DNA QIAcube HT Kit	For 480 preps: QIAamp plates, QIAGEN Proteinase K, Buffers	51331
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
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
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

* 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.
Consumables		
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
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
Reagent Trough (with Lid), 170 mL	Box of 20 plus lid; liquid reservoirs	990556
Reagent Trough (with Lid), 70 mL	Box of 10 plus lid; liquid reservoirs	990554
Buffer ATL (200 mL)	200 mL Tissue Lysis Buffer for 1000 preps	19076

For a complete list of consumables, 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. If you are using QIAcube HT 4.17 Software, download the corresponding protocol at **www.qiagen.com/HB-1569**

Further information

- *QIAamp 96 DNA QIAcube HT Handbook:* **www.qiagen.com/HB-2158**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for the purification of gDNA from blood, cells, and soft tissues, such as biopsies, mouse tails, brain, liver, and muscle using the QIAamp 96 DNA QIAcube HT Kit with the QIAcube HT Prep Manager Software.
- For processing tissue samples, you will also need Collection Microtube Racks (cat. no. 19560) and Collection Microtube Caps (cat. no. 19566).
- Prepare Buffers ACB, AW1, and AW2 according to the instructions in the *QIAamp 96 DNA QIAcube HT Handbook*.
- For processing blood and cell samples, prepare a mixture of Buffer VXL and proteinase K immediately before starting the run according to Table 1.

  Note: If you use tissues, proteinase K is already used during sample disruption. In this case, load only an appropriate amount of Buffer VXL as shown on the virtual worktable.

1. Start the QIAcube HT Prep Manager Software. Click on the **Home** icon in the main toolbar to access the home screen.
2. Select **QIAamp 96 DNA** from the **Create Experiment** list. Follow the instructions in the wizard and fill in all required fields.

3. In the **Setup** step, select **Sample type** and **Pre-treatment** for documentation.
4. Select the **QIAamp DNA protocol**. For information about optional steps and advanced options see the kit handbook.
5. Define samples in the **Labware selection** step.
6. 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.
7. Follow the instructions on the virtual worktable to prepare the instrument.
8. Add the volume of sample as indicated on the virtual worktable to the selected S-Block wells.
9. Save the experiment by clicking the **Save** button in the button bar.
10. Click the **Start run** button to start the run.

    Important: If the optional Vacuum performance check has been selected, the software will show a dialog that needs to be confirmed after defined vacuum steps.
11. When the protocol is complete, cover the elution plate (EMTR) with the lid and remove it from the elution chamber.

    Note: If using TopElute fluid, there may be 2 liquid phases in the elution microtubes. TopElute fluid will be the top layer over the elution buffer.
12. Create a report (if required).
13. Follow the cleaning procedure.

Table 2. Buffer VXL mixture preparation.

Samples	24	32	40	48	56	64	72	80	88
Buffer VXL (mL)	2.48	3.2	4.0	4.72	5.44	6.16	6.88	7.68	8.4
Proteinase K (µL)	620	800	1000	1180	1360	1540	1720	1920	2100

Document Revision History

Revision	Description
December 2017	Corrected certain protocol steps. Notes on off-board lysis were deleted. Omitted repetitive text. Updated ordering information.
November 2022	Added a footnote in Kit Contents indicating sodium azide as a preservative ingredient in Buffer AW2. Layout and editorial changes.

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