November 2022

QIAamp® 96 DNA QIAcube® HT Handbook

For automated purification of total DNA from blood and tissue using QIAcube® HT and QIAxtractor® instrument

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 98 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), and TopElute Fluid. 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.

The QIAamp 96 DNA QIAcube HT Kit includes a ready-to-use proteinase K solution in a specially formulated storage buffer. QIAGEN Proteinase K is stable for at least 1 year after delivery when stored at room temperature (15–25°C). For storage for >1 year, 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 	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.

24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from: CHEMTREC

USA & Canada ■ Tel: 1-800-424-9300

Outside USA & Canada ■ Tel: +1-703-527-3887 (collect calls accepted)

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). 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.

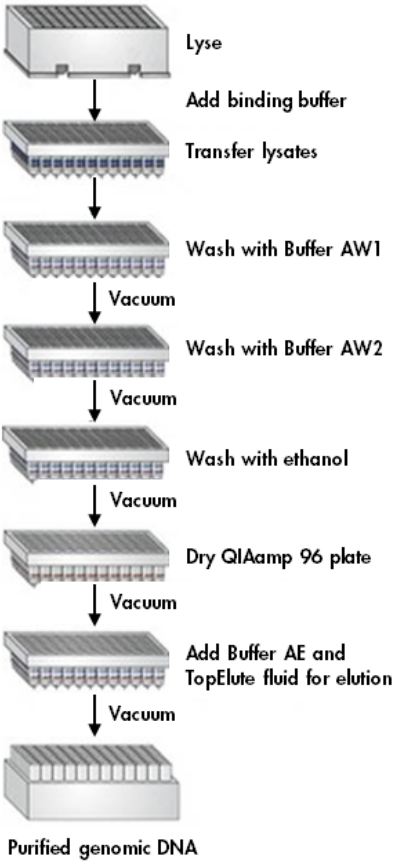
The fully automated procedure requires <2 hours to process 96 samples, with no hands-on time. Turnaround time between consecutive runs is about 10 minutes.

Principle and procedure

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



Description of protocols

Different protocols in this handbook provide detailed instructions to use the QIAamp 96 DNA QIAcube HT Kit for purification of total DNA.

The protocol “Protocol: Purification of Genomic DNA from Whole Blood Cell Samples”, page 19, is for the purification of DNA from blood, including blood with nucleated erythrocytes, or from cultured animal or human cells.

The protocol “Protocol: Purification of Genomic DNA from Tissue Samples”, page 26, is for the purification of DNA from human or animal tissues, including rodent tails.

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)
- QIAcube HT instrument
- QIAcube HT Software version 4.17 or higher
- To process dedicated QIAcube HT Kits on the QIAxtractor instrument, QIAcube HT Software version 4.17 or higher is needed, together with the Accessories Pack, QXT.†
- Reagent troughs
- Vortexer
- Ethanol (96–100%)*

For blood and cultured cells

- PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)

Plasticware for purification from tissue samples

- Collection Microtube Racks (cat. no. 19560)†
- Collection Microtube Caps (cat. no. 19566)†

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
† For ordering, see "Ordering Information" on page 38.

TissueLyser system

The TissueLyser system 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 gives yields comparable to or better than those 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 provides simultaneous disruption for high-throughput processing of a variety of human and animal tissues.

The TissueLyser system includes a number of different accessories for ease of use with different 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 39.

Important Notes

Amount of 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.

Amount of 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.

Preparing reagents

Important: Sufficient reagents are supplied to isolate DNA from 5 x 96 blood or buffy coat samples. If <96 samples are processed in each run, additional reagents must be purchased to process the same number of samples in total.

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 40 mL of isopropanol (100%), as indicated on the bottle. 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 130 mL of ethanol (96–100%), as indicated on the bottle. 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 160 mL of ethanol (96–100%), as indicated on the bottle. 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.

TopElute does not evaporate and can be stored in the reagent trough.

Assembling the vacuum chamber

Figure 1 illustrates the assembly of the vacuum chamber when using QIAamp 96 plates.

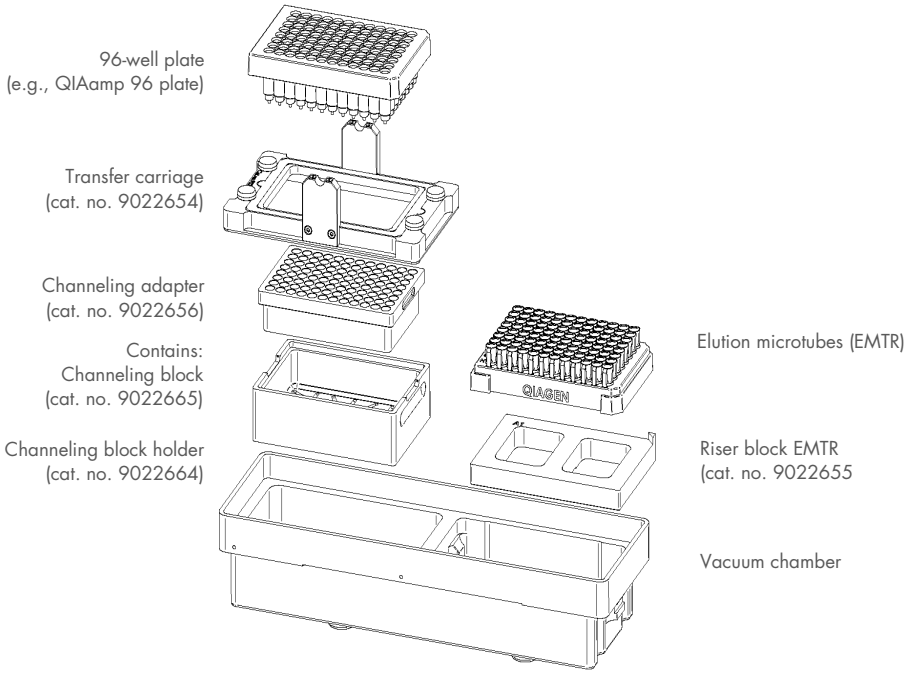


Figure 1. Assembling the vacuum chamber.

All QIAcube HT instruments are delivered with the vacuum chamber components for dedicated QIAcube HT Kits.

> **Important:** If you use a QIAxtractor instrument, ensure that only parts from the Accessories Pack, QXT (black parts) are used. See "Ordering Information", page 36.

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.

Optional features

Processing of fewer than 96 samples per run

If processing fewer than 96 samples, reuse 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.
- Remove unused Elution Microtubes from the EMTR in rows of eight tubes.

Sample data input, data tracking, and LIMS connection

In the software environment, information about an item can be seen in the right-hand panel (for example, click on **A1: Reaction**). See Section 5.11 in the *QIAcube HT User Manual* for more information.

In the software environment, information about an item can be seen in the right-hand pane (to open the dialog, click on **A1: Reaction**).

See Section 5.11 in the *QIAcube HT User Manual* for more information or contact QIAGEN Technical Service for a detailed example.

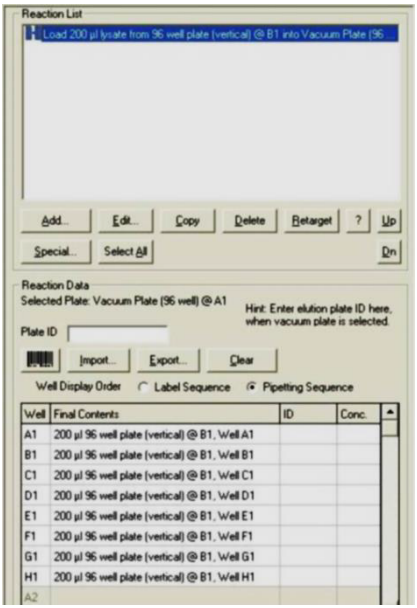


Figure 2. Example for right-hand pane information.

Sample descriptions can be imported, inserted manually, or inserted using a handheld barcode scanner.

The field **Plate ID** can be used for the unique number that is provided on each EMTR RS plate.

A post-run report is generated for each run and can be used for quality management purposes. It is shown after each run and is automatically saved in the **Reports** subdirectory of the **Data** directory (default location is **C:\Program Files\QIAcubeHT\Data**).

Protocol: Purification of Genomic DNA from Whole Blood Cell Samples

This protocol is for purification of genomic DNA from up to 96 samples of fresh or frozen whole blood, buffy coat, or cell samples.

Important points before starting

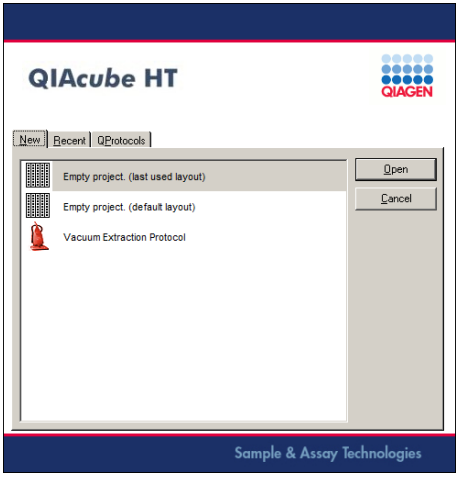
- Read "Important Notes" on page 13.
- Check for precipitates in reagents. If a reagent contains precipitates, incubate at 37°C with gentle shaking to dissolve precipitates. Avoid vigorous shaking which causes foaming.

Things to do before starting

- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).
- Check that Buffer ACB, Buffer AW1, and Buffer AW2 have been prepared according to the instructions in "Preparing reagents" (page 14).
- Ensure that the relevant version of the QIAamp 96 DNA QIAcube HT.QSP run file is installed.
  QIAcube HT protocol files (file extension ***.QSP**), 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 Software version 4.17 or higher is installed. This is mandatory to process QIAamp 96 plates on the QIAcube HT and QIAxtractor.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.
- If the volume of the samples is less than 200 µL, add PBS or 0.9% NaCl to a final volume of 200 µL.

Procedure

1. Place the tip discard chute on the worktable so that the chute is over the tip disposal box. Ensure that the tip discard chute is open and unblocked. Remove the UV protective cap from the tip chute. Make sure that the tip disposal box is empty and that the opening is aligned with the tip discard chute.
2. Switch on the instrument. The switch is located at the back of the instrument, on the lower left.
3. Launch the QIAcube HT Software. The following screen appears.



Note: If the QIAcube HT Software is already open, click in the toolbar.

4. Select the **QProtocols** tab. All Q Protocols that are saved in the appropriate **QProtocols** folder will be listed.
5. To open the run file, select the Q Protocol and then click **Open**. Alternatively, double-click on the Q Protocol.
6. A **Protocol Description** of the selected Q Protocol will be displayed and the QIAGEN Protocol icon will appear in the toolbar.
7. Check that the Q Protocol meets your requirements, and then click **Close**.

   Note: To view the Q Protocol information box again, click on the icon in the toolbar.

8. Click  in the toolbar. The **Configuration (1)** step of the **Vacuum extraction** wizard opens. This wizard displays protocol parameters. For information about adjusting the parameters, see the *QIAcube HT User Manual*.
9. Select the appropriate number of samples arranged in columns in the 96-well plate. Ensure the **Turn the HEPA filter on automatically** option is checked, and click **Jump to End**.

   Reagent and consumable lot numbers can be entered in the **Configuration (1)** window for tracking. The **Jump to End** button is located at the bottom left of the **Configuration (1)** window.

   The **Wizard Summary** window opens. The information in this window can be printed for documentation purposes.
10. Confirm the protocol by clicking **Finish**. The wizard closes. The QIAcube HT Software calculates the reagent volumes and the number of tips required to complete the protocol. These values are displayed with the worktable layout in the QIAcube HT workspace. For detailed information, see the *QIAcube HT User Manual*.
11. Ensure that there are sufficient numbers of tips for all steps, at least up to and including lysate transfer. Ensure that tip boxes are placed in the indicated positions, and that the lids have been removed from the tip boxes. 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 pre-run check. For more information, see the *QIAcube HT User Manual*.

    In the software environment, click on a tip in any tip position to open the **tip info** preview.
12. Prepare the vacuum chamber as described in "Assembling the vacuum chamber", page 15. See the *QIAcube HT User Manual* for more information.

    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: Be 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.

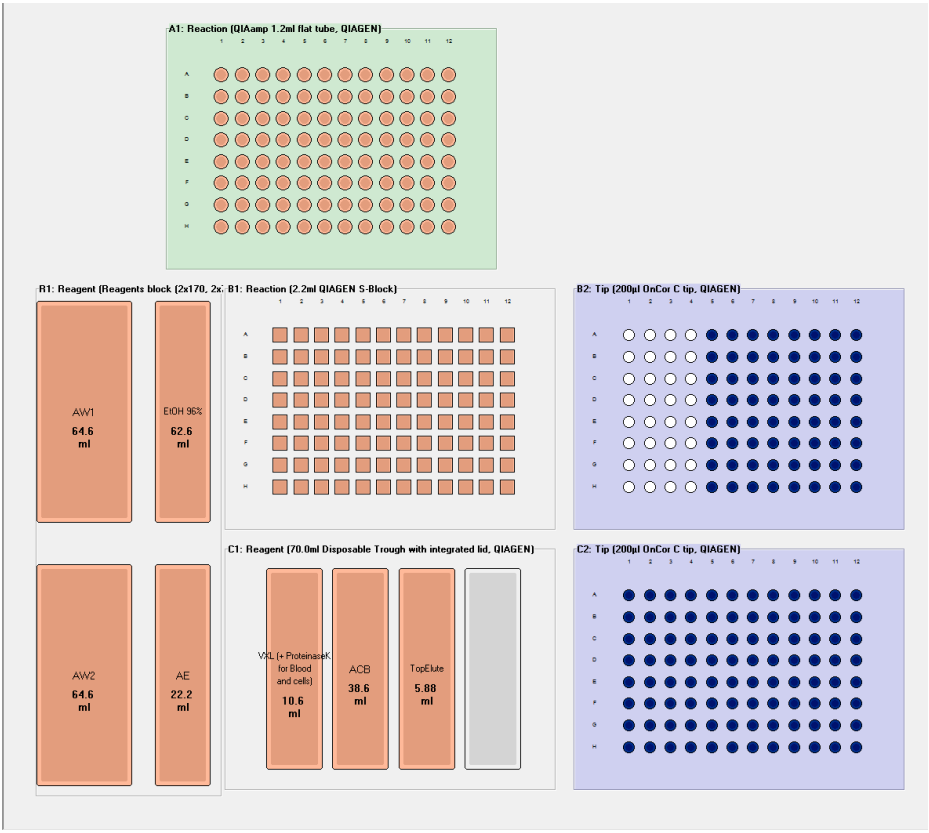
13. Add 200 µL of sample to the selected S-Block wells. Place the S-Block in the B1 position of the QIAcube HT worktable.

    Note: If the volume of a sample is less than 200 µL, add PBS to a final volume of 200 µL.

    Note: Samples are processed in columns of 8 wells. We recommend covering unused wells during pipetting for subsequent reuse of the S-Block. If fewer than 8 samples per column are to be processed, unused wells in the column must contain water or buffer. The volume added to unused wells should be the same as the sample volume to avoid foaming.

    Optional: Add 4 µL RNase A (100 mg/mL) to each sample if RNA-free genomic DNA is required.

14. Transfer the indicated volumes of all reagents, except Buffer VXL mixture, into the corresponding reagent troughs, close the lids, and place them on the indicated positions on the worktable.



15. Prepare the indicated volume of VXL mixture, and mix well.

    Important: Prepare VXL mixture immediately before starting the run.

Table 1. Preparation of Buffer VXL and QIAGEN Proteinase K mixture

Samples	24	32	40	48	56	64	72	80	88	96
Buffer VXL (mL)	2.72	3.36	4.0	4.64	5.28	5.92	6.56	7.2	7.84	8.48
QIAGEN Proteinase K (mL)	680	840	1000	1160	1320	1480	1640	1800	1960	2120

16. Start the run immediately by clicking  . The pre-run checklist appears.

17. Perform the pre-run check. Confirm that the worktable is set up correctly (instrument does not perform checks for all items). Check the box to the left of the items. A pre-run report can be saved for documentation purposes by clicking  .
18. After completing the pre-run check, close the instrument hood and click **OK**. The **OK** button is disabled until all pre-run checklist items have been checked.
19. Click **Cancel** when the **Save as** dialog box appears. The protocol run begins.

    Important: At the beginning of each run, an open circuit test and a plate detection test is 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 place it correctly. After adjusting the position, click **Retry** to initiate the tests again.
20. Cover the elution plate (EMTR) with the lid and remove from the elution compartment, when the protocol is complete. See the *QIAcube HT User Manual* for detailed instructions.

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

Cleaning the instrument after completing a run

1. Discard racks containing only used tips.
2. Discard leftover reagents.

   We recommend not reusing reagents in multiple runs. Reagents provided are sufficient for at least 5 runs of 96 samples.

   Do not clean the trough containing TopElute Fluid with water. Clean with a dry, lint-free cloth only.
3. Discard the S-Block or keep partially used blocks for reuse.
4. Remove the transfer carriage and discard the QIAamp 96 plate or keep partially used QIAamp 96 plates for reuse.
5. Clean the carriage, channeling-block, channeling-block holder, and tip chute.

6. With a damp cloth, clean any spilt reagent on the instrument worktable or vacuum chamber.

   For all further cleaning and maintenance operations, see Section 7 of the *QIAcube HT User Manual*.

7. Turn on the UV lamp to decontaminate the worktable by clicking  . See the *QIAcube HT User Manual* for detailed instructions.

Protocol: Purification of Genomic DNA from Tissue Samples

This protocol is for purification of genomic DNA from up to 96 samples of fresh or frozen tissue samples (maximum of 20 mg), such as biopsies, mouse tails, brain, liver, and muscle.

Important points before starting

- Read "Important Notes" on page 13.
- 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.
- For this protocol, Collection Microtube Racks (cat. no. 19560) and Collection Microtube Caps (cat. no. 19566) need to be ordered separately.

Things to do before starting

- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).
- Check that Buffer ACB, Buffer AW1, and Buffer AW2 have been prepared according to the instructions in "Preparing reagents" (page 14).
- Ensure that the relevant version of the QIAamp 96 DNA QIAcube HT.QSP run file is installed.
- QIAcube HT protocol files (file extension ***.QSP**), 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 Software version 4.17 or higher is installed. This is mandatory to process QIAamp 96 plates on the QIAcube HT and QIAxtractor.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

Tissue: manual treatment

For homogenization with the TissueLyser, see "Tissue: disrupting and homogenizing with the TissueLyser" below.

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.

   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.

   Optional: Tissue disruption using the TissueLyser will facilitate the digestion process and shorten the time of digestion (see below).
2. Prepare a proteinase K – Buffer ATL working solution containing 20 µL proteinase K stock solution and 180 µL ATL per sample, and mix by vortexing.
3. Immediately pipette 200 µL working solution into each well containing the cell or tissue samples. Seal the microtubes properly with collection microtube caps.
4. Place a clear cover of collection microtubes over each rack, and mix by inverting the rack of collection microtubes.
5. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge. Proceed to lysis of tissue (see below).

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

Tissue: disrupting and homogenizing with the TissueLyser

1. Place 5 mm stainless steel beads into the collection microtubes (1 bead per tube).

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

2. Determine the amount of tissue. Do not use more than 20 mg tissue in 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.

   Weighing tissue is the most accurate way to determine the amount. 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 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. Proceed to lysis of tissue (see 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 vigorously shake the racks up and down for 15 s.
3. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

   Optional: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL). Close the collection microtubes with fresh caps, mix by shaking vigorously, and incubate for

5 min at room temperature. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

Procedure for tissue samples

1. Place the tip discard chute on the worktable so that the chute is over the tip disposal box.

   Ensure that the tip discard chute is open and unblocked. Remove the UV protective cap from the tip chute.

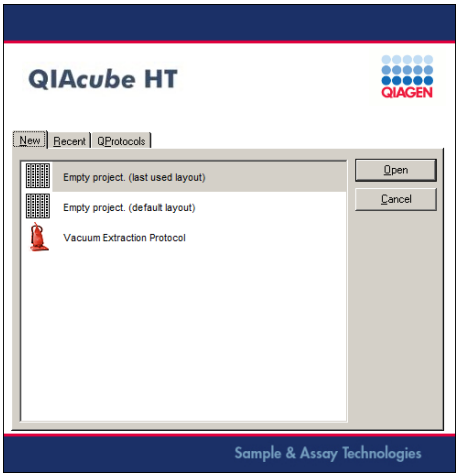
   Make sure that the tip disposal box is empty and that the opening is aligned with the tip discard chute.

2. Switch on the instrument. The switch is located at the back of the instrument, on the lower left.

3. Launch the QIAcube HT Software.

   Note: If the QIAcube HT Software is already open, click  in the toolbar.

4. The following screen appears.



5. Select the **QProtocols** tab. All Q Protocols that are saved in the appropriate **QProtocols** folder will be listed.

6. To open the run file, select the **Q Protocol** and then click **Open**. Alternatively, double left-click on the **Q Protocol**.
7. A **Protocol Description** of the selected Q Protocol will be displayed and the QIAGEN Protocol icon will appear in the toolbar.
8. Check that the Q Protocol meets your requirements, and then click **Close**.

   Note: To view the Q Protocol information box again, click on the  icon in the toolbar.
9. Click  in the toolbar.

   The **Configuration (1)** step of the **Vacuum extraction** wizard opens. This wizard displays protocol parameters. For information about adjusting the parameters, see the *QIAcube HT User Manual*.
10. Select the appropriate number of samples arranged in columns in the 96-well plate. Ensure the **Turn the HEPA filter on automatically** option is checked, and click **Jump to End**.

    Reagent and consumable lot numbers can be entered in the **Configuration (1)** window for tracking.

    The **Jump to End** button is located at the bottom left of the **Configuration (1)** window. The **Wizard Summary** window opens. The information in this window can be printed for documentation purposes.
11. Confirm the protocol by clicking **Finish**. The wizard closes.

    The QIAcube HT Software calculates the reagent volumes and the number of tips required to complete the protocol. These values are displayed with the worktable layout in the QIAcube HT workspace. For detailed information, see the *QIAcube HT User Manual*.
12. Ensure that there are sufficient numbers of tips for all steps, at least up to and including lysate transfer. Ensure that tip boxes are placed in the indicated positions, and that the lids have been removed from the tip boxes.

    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 pre run check. For more information, see the *QIAcube HT User Manual*.

In the software environment, click on a tip in any tip position to open the **tip info** preview.

13. Prepare the vacuum chamber as described in "Assembling the vacuum chamber", page 15. See the *QIAcube HT User Manual* for more information.

    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 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.

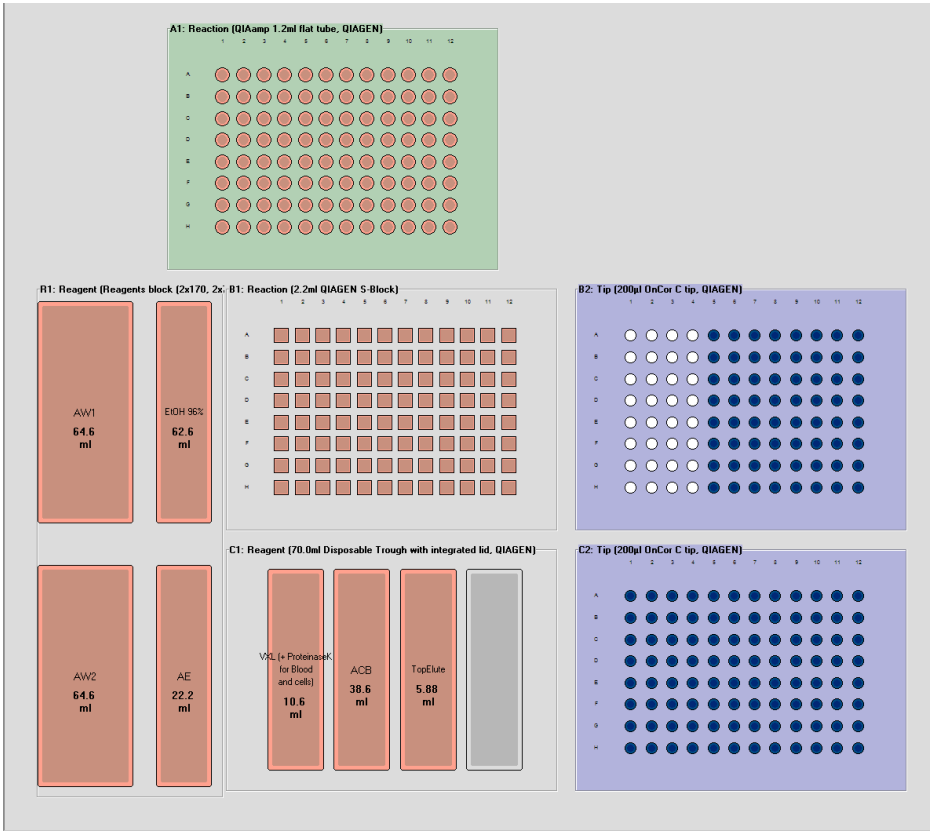
14. Carefully remove caps and transfer lysate into new S-Block.

15. Add 200 µL of sample to the selected S-Block wells. Place the S-Block in the B1 position of the QIAcube HT worktable.

    Note: Samples are processed in columns of 8 wells. We recommend covering unused wells during pipetting for subsequent reuse of the S-Block.

    Note: If fewer than 8 samples per column are to be processed, unused wells in the column must contain water or buffer. The volume added to unused wells should be the same as the sample volume to avoid foaming.

16. Transfer the indicated volumes of all reagents into the corresponding reagent troughs, close the lids, and place them on the indicated positions on the worktable.



17. Start the run immediately by clicking  . The pre-run checklist appears.
18. Perform the pre-run check. Check the state of the worktable items. Confirm that the worktable is set up correctly (instrument does not perform checks for all items). Check the box to the left of the items.

    A pre-run report can be saved for documentation purposes by clicking  .
19. After completing the pre-run check, close the instrument hood and click **OK**. The **OK** button is disabled until all pre-run checklist items have been checked.
20. Click **Cancel** when the **Save as** dialog box appears.

Optional: Save the run file with a unique file name. See the *QIAcube HT User Manual* for more details.

21. The protocol run begins.

    Important: At the beginning of each run, an open circuit test and a plate detection test is 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 place it correctly. After adjusting the position, click **Retry** to initiate the tests again.

22. Cover the elution plate (EMTR) with the lid and remove from the elution compartment, when the protocol is complete. See the *QIAcube HT User Manual* for detailed instructions.

    Two liquid phases may be found in the Elution Microtubes. TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

23. See page 24 for the procedure on “Cleaning the instrument after completing a run”.

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
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 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 mixing samples immediately before a run is started.
c) Insufficient sample lysis	QIAGEN Proteinase K was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh proteinase K (see "Storage", page 5). For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. For this purpose, an off-board-lysis protocol is available.
d) 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.
e) Buffer VXL – QIAGEN Proteinase K mixture mixed insufficiently	Mix well by pipetting with a large pipette.
f) DNA degraded	DNA may have been degraded by DNases in the starting material. Ensure that samples are processed quickly 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.

	Comments and suggestions
g) Samples not equilibrated to room temperature	Using cold samples can lower the lysis temperature, leading to incomplete sample lysis.

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.

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 for possible reasons. Increase the amount of eluate added to the reaction if possible. If necessary, concentrate the DNA under vacuum, or repeat the procedure using fresh samples.
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.

	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) Instrument failure/cancelled run	It is possible to restart the protocol from the last successful step. The post-run report indicates the step where the error occurred. It is often possible to delete all steps before the indicated step in the right-hand pane and to restart the run from this point. Be sure that all parts and buffers are in the correct position.
c) Blocked membranes	a) Sample not well lysed. If liquid is still visible after vacuum, remove 500 µl using a pipette. Then, scrape the surface of the membrane with a fresh pipette tip to relocate any solid particles that may block the membrane. Take care not the damage the membrane. If there is still no liquid flow, pipette all liquid from the well and proceed with the run. After adding Buffer AW2, open the hood to pause the run. Check if the well is still blocked. If so, remove all liquid using a pipette and mark the well as invalid. 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) and prolong the digestion step. 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.
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

* 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.

Ordering Information

Product	Contents	Cat. no.
QIAamp 96 DNA QIAcube HT Kit	For 480 preps: QIAamp plates, QIAGEN Proteinase K, Buffers	51331
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
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 (available separately)*	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 (available separately)†	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
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 Pack, QXT*	Upgrade kit for QIAxtractor instrument; Adapter set to use dedicated QIAcube HT kits on the QIAxtractor Contains: Transfer Carriage (9022654), Riser Block EMTR (9022655), and Channeling Adapter (9022656)	9022649
Accessories		
Collection Microtubes (racked)	(10 x 96) Nonsterile polypropylene tubes, 960 in racks of 96	19560
Collection Microtube Caps	960 in strips of 8, For capping collection microtubes and round-well blocks	19566
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

Product	Contents	Cat. no.
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.

Document Revision History

Revision	Description
December 2013	Added plasticware to Equipment and Reagents to Be Supplied by User. Updated Storage and Important Notes. Troubleshooting Guide now covers blocked membranes issue. Added accessories in Ordering Information. Updated protocols.
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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