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QIAamp[®] 96 Virus QIAcube[®] HT Handbook

For automated purification of viral RNA and
DNA from human samples using QIAcube HT
and QIAcube HT Prep Manager Software

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Kit Contents

QIAamp 96 Virus QIAcube HT Kit	(5)
Catalog no.	57731
Number of preps	480
QIAamp 96 plates	5
Buffer ACL*	1 x 220 ml
Buffer ACB*† (concentrate)	3 x 60 ml
QIAGEN Proteinase K	3 x 6 ml
Carrier RNA (poly A)	2 x 310 µg
Buffer ATL	2 x 50 ml
Buffer AW1 *‡ (concentrate)	1 x 190 ml
Buffer AW2‡ (concentrate)	1 x 127 ml
Buffer AVE§	1 x 125 ml
TopElute Fluid	1 x 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.

§ **CAUTION:** 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

Storage

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

Freeze Carrier RNA dissolved in Buffer AVE immediately in aliquots at –30 to –15°C. Do not subject aliquots of carrier RNA to more than 3 freeze–thaw cycles. Carrier RNA dissolved in Buffer AVE is stable at room temperature (15–25°C) for up to 48 hours (see “Preparing reagents and instrument” on page 17 for further information).

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 Virus QIAcube HT Kit is intended for the automated extraction of viral RNA and DNA from human whole blood, serum, plasma, body fluids, swabs, washes, tissue and stool using the QIAcube HT instrument. The QIAamp 96 Virus 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 ACL and Buffer ACB contain guanidine thiocyanate and Buffer AW1 contains guanidine hydrochloride, 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 Virus QIAcube HT Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAamp 96 Virus QIAcube HT Kit uses well-established technology to enable efficient purification of viral RNA and DNA from a broad range of sample types (see Table 1 on page 9).

The kit combines the selective binding properties of a silica-based membrane with a high-throughput 96-well format, and is designed for fully-automated, simultaneous processing of 96 samples on the QIAcube HT. Purified nucleic acids are eluted in Buffer AVE, and are free of proteins, nucleases and other impurities, ready for use in downstream applications. The kit is not intended for host RNA or host DNA preparation.

Principle and procedure

Samples are lysed under highly denaturing conditions at room temperature in the presence of QIAGEN proteinase K and Buffer ACL, which together ensure the inactivation of nucleases. Adding Buffer ACB adjusts the binding conditions for the co-purification of DNA and RNA. The lysate is then transferred to a QIAamp 96 plate. During vacuum, nucleic acids are adsorbed onto the silica membranes while contaminants pass through. Three efficient wash steps remove the remaining contaminants and enzyme inhibitors, and nucleic acids are eluted in Buffer AVE.

Performance is not guaranteed for every combination of starting material and virus species and must be validated by the user. Some samples may require a pretreatment (see Table 1 on page 9).

Description of protocols

Samples will either directly undergo nucleic acid purification, or undergo pretreatment followed by nucleic acid purification.

Many sample types can be directly processed without pretreatment. However, depending on the starting material, a sample pretreatment may be needed. Table 1 provides an overview of pretreatment protocols suited to different starting materials. This handbook includes the following protocols:

- QIAamp Virus Protocol (page 21)
- Sample Pretreatment Protocols (pages 34–39)

Nucleic acid purification protocol

The protocol “QIAamp Virus Protocol”, page 21, is optimized for purification of viral RNA and DNA from up to 200 µl of fluid material. Suitable starting materials for direct processing using this method include:

- Cell-free body fluids such as plasma, serum and CSF
- Whole blood

Pretreatments

The various pretreatments included in this handbook are optimized for specific combinations of starting material. The choice of pretreatment depends on the workflow focus, and is to be followed by nucleic acid purification. Table 1 on page 9 summarizes the pretreatments and their applications.

Some of the pretreatments may require additional components (see pages 10–11).

Table 1. Pretreatment protocols for various sample types

Sample	Name	Page
Fluids (e.g., plasma and serum*, cell-free body fluids†, whole blood†‡)	No pretreatment necessary; proceed directly with “QIAamp Virus Protocol”	21
Respiratory samples and sputum§	Pretreatment B1 for Respiratory Samples	34
Liquid transport media	Pretreatment B2 for Liquid Transport Media	35
Urine	Pretreatment B3 for Urine	35
Dried blood spots	Pretreatment B4 for Dried Blood Spots	36
Swabs and buccal cells	Pretreatment B5 for Swabs and Buccal Cells	36
Stool suspensions	Pretreatment F1 for Stool Suspensions	37
Tissues (e.g., liver, spleen, kidney, lymph node)	Pretreatment T1 Mechanical Disruption of Tissue Pretreatment T2 for Enzymatic Digestion of Tissue¶	37 38

*Samples can be fresh or frozen, provided they have not been frozen and thawed more than once.

† Includes amniotic fluid, cerebrospinal fluid, ocular fluid, synovial fluid and pleural fluid.

‡ Includes samples containing EDTA or citrate.

§ Includes nasopharyngeal aspirates (NPA), bronchoalveolar lavage (BAL) fluid, sputum and nasal and throat swabs.

¶ Not suitable for viral RNA as the lysis conditions do not sufficiently conserve RNA integrity.

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:

- Pipets and disposable pipet tips with aerosol barriers (20–1000 µl)
- Isopropanol
- Ethanol (96–100%)*
- Phosphate-buffered saline (PBS) may be required for sample dilution
- QIAcube HT Instrument
- QIAcube HT Prep Manager Software
- QIAcube HT Reagent troughs
- Vortexer

Pretreatment B1 – for respiratory samples

- Sputasol (Oxoid Limited) and 37°C water bath, or
- NAC buffer (10 g N-acetylcysteine per liter of 0.9% NaCl solution), or
- PBS or Buffer AE (cat. no. 19077), DTT and 37°C water bath

Pretreatment B2, B3, B4 and B5 – for liquid transport media, urine and dried blood spots

- Buffer ATL (cat. no. 19076)
- QIAGEN Proteinase K (cat. nos. 19131 or 19133)

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

Pretreatment F1 — stool samples

- 0.9% NaCl solution

Pretreatment T1 – mechanical disruption of tissue

- TissueLyser II (QIAGEN, cat. no. 85300) with a TissueLyser II Adapter Set 2 x 24 (QIAGEN, cat. no. 69982), or TissueLyser LT (QIAGEN, cat. no. 85600) with the TissueLyser LT Adapter for 12 tubes (QIAGEN, cat. no. 69980), or other bead-mill homogenizer*
- 5 mm stainless steel beads (cat. no. 69989)
- Buffer ATL (cat. no. 19076)
- QIAGEN Proteinase K (cat. nos. 19131 or 19133)

Pretreatment T2 – enzymatic digestion of tissue

- Thermoshaker suitable for 2 ml collection tubes
- Buffer ATL (cat. no. 19076)
- QIAGEN Proteinase K (cat. nos. 19131 or 19133)

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Starting materials

Do not overload the QIAamp membrane, as this can lead to impaired nucleic acid extraction and/or performance in downstream assays. For samples with very high host nucleic acid contents (e.g., for certain tissues, such as spleen or blood samples with highly increased cell counts), use less than the maximum amount of sample recommended in the protocol or pretreatments. In some downstream applications such as PCR and RT-PCR, very high background concentrations of nucleic acids may impair the reaction. Use appropriate controls (e.g., an internal control) to verify successful PCR amplification.

Avoid transferring solid material to the S-Block that could reduce flow through the membrane (e.g., blood clots, solid tissue, swab fibers, etc.). When working with difficult samples, use a vacuum performance step to check if all liquid has passed the membrane. See “Troubleshooting Guide” on page 40 and the *QIAcube HT User Manual* for guidance.

Highly viscous fluids may require a treatment to reduce their viscosity to allow for efficient extraction of viral nucleic acids.

Avoid repeated thawing and freezing of samples since this may reduce nucleic acid yield and quality.

Serum, plasma, other body fluids and swab media

Up to 200 µl serum, plasma, other body fluid or swab media supernatant can be processed.

Carrier RNA should be used in the nucleic acid purification protocol to prevent the loss of nucleic acids during the procedure (see page 16 for information about the use of carrier RNA).

The processing of samples with very high inhibitor contents, such as urine or stool, may require a reduction in sample input volume and/or an extra pretreatment to remove inhibitors. To reduce the input volume, use 25–50 µl of the sample and adjust the volume to 200 µl with PBS or 0.9% NaCl.

Plasma and serum samples

After collection, centrifugation and phase separation, plasma or serum can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –30 to –15°C or –70°C in aliquots is recommended. Frozen plasma or serum samples must not be thawed more than once. Repeated freeze–thawing leads to reduced viral titers and therefore reduced yields of viral nucleic acids.

Freezing of samples can lead to denaturation and precipitation of proteins that may aggregate on the QIAamp membrane. If cryo-precipitates are visible, they can be pelleted by centrifugation at 6800 x g for 3 minutes. The cleared supernatant should be transferred to a new sample tube without disturbing the pellet and processed immediately. This step will not reduce viral titers.

Swabs

Swabs may be processed on the same day as collection or stored for future processing. While storage at –30 to –15°C is recommended, DNA of suitable quality for single-copy gene amplification has been documented from swabs stored at room temperature for 24 months.

Alternatively, samples can be collected using plastic swabs with cotton or Dacron® tips. Puritan® applicators with plastic shafts and cotton or Dacron tips are available from Hardwood Products Company (www.hwppuritan.com, cat. nos. 25-806 1PC and 25-806

1PD) and from Daigger (www.daigger.com, cat. nos. EF22008D and EF22008DA).^{*} Nylon cytology brushes and other swab types may also be used.

Note: Solid pieces remaining in the sample fluid may aggregate on the QIAamp membrane, which may decrease nucleic acid yield.

Whole blood

Blood samples treated with EDTA or citrate as anticoagulant can be used for nucleic acid purification. Samples can be either fresh or frozen, provided that they have not been frozen and thawed more than once. After collection, whole blood samples can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at –30 to –15°C or –70°C.

We recommend using 50–200 µl blood per sample. Typically, 200 µl of blood can be used with most blood samples. However, highly elevated cell counts due to inflammatory or neoplastic diseases may strongly increase the host nucleic acid content of a sample. In this case, reduction of sample input to 50 µl may improve results in downstream assays, particularly in RT-PCR. If using less than 200 µl blood, adjust the sample volume to 200 µl with PBS or 0.9% NaCl.

Dried blood spots

Drying blood on filter paper (e.g., Whatman FTA Cards) is an effective form of storage, and samples prepared in this manner are less expensive and safer to transport. A disc (3 mm diameter) punched out from filter paper stained with dried blood contains white blood cells from approximately 5 µl whole blood.

^{*} This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Tissues

When working with tissue samples, mechanical or enzymatic disruption of the tissue structure is the prerequisite for liberation of cells, subsequent release of nucleic acids, and membrane permeability of the material.

Different tissue types can vary widely with regard to texture and rigidity, cell types and content of host nucleic acids and inhibitory substances. In addition, the localization of viral nucleic acids in the tissue may vary depending on tissue type, virus and stage of infection.

Up to 5 mg of fresh or frozen tissue can be used as a starting amount. For higher amounts of tissue it might be necessary to check whether this amount of tissue can still be processed. Furthermore we recommend enabling the vacuum performance check during the vacuum step to ensure that the liquids have completely passed through the membrane before the protocol proceeds.

Yields of nucleic acids

For samples containing a low amount of cells (e.g., serum and plasma), the yield of nucleic acids obtained can be below 1 µg and is therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with carrier RNA may contain much more carrier RNA than target nucleic acids. The QIAamp 96 Virus QIAcube HT Kit recovers total nucleic acids. Therefore, cellular DNA and RNA and circulating cell-free nucleic acids will be co-purified along with viral RNA and DNA (and bacterial nucleic acid if present) and cannot be distinguished using spectrophotometric measurements. We recommend using quantitative amplification methods such as quantitative real-time PCR or real-time RT-PCR to determine viral nucleic acid yields.

Using carrier RNA and internal controls

Carrier RNA

We recommend adding carrier RNA to fluids containing low amounts of cells such as serum, plasma, swab media and wash fluid. This enhances adsorption of viral RNA and DNA to the silica membranes, which is especially important when the target molecules are not abundant. In addition, an excess of carrier RNA reduces the chances of viral RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergents in the lysis buffer. Not using carrier RNA may decrease the recovery of viral nucleic acids.

Internal control

Using the QIAamp 96 Virus QIAcube HT Kit in combination with commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control RNA or DNA should be added together with the carrier RNA to the lysis buffer (see Table 2 on page 26). Refer to the assay manufacturer's instructions in order to determine the optimal concentration.

For the standard protocol, it is recommended to add 5 µl internal control solution. Using a concentration other than that recommended may reduce amplification efficiency.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing the purified viral RNA and DNA at 2–8°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at –30 to –15°C, or even –70°C in the case of RNA.

Handling RNA

RNases are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and only minute amounts are sufficient to

destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Preparing RNA

When preparing viral RNA, work quickly during the manual steps of the procedure. If you have not previously worked with RNA, read Appendix A on page 43 before starting. Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase inhibitors, it will not actively inhibit RNases introduced by inappropriate handling. Extreme care should be taken to avoid contamination with RNases when handling Buffer AVE.

Preparing reagents and instrument

Carrier RNA stock solution

For use, lyophilized carrier RNA should first be dissolved in Buffer AVE. Add 1550 μ l Buffer AVE to the tube containing 310 μ g lyophilized carrier RNA to obtain a stock solution of 0.2 μ g/ μ l. Add this solution to Buffer ACL as described in Table 2 on page 26. Unused carrier RNA dissolved in Buffer AVE should be frozen in aliquots at -30 to -15°C . Aliquots of carrier RNA should not be subjected to more than 3 freeze-thaw cycles.

QIAGEN Proteinase K

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

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

Buffer 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.

Handling Buffer AVE

Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase-degrading chemicals, it will not actively inhibit RNases introduced by inappropriate handling. For RNA applications, when handling Buffer AVE, avoid contamination with RNases. Follow general precautions for working with RNA, such as frequent change of gloves and keeping tubes closed whenever possible.

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.

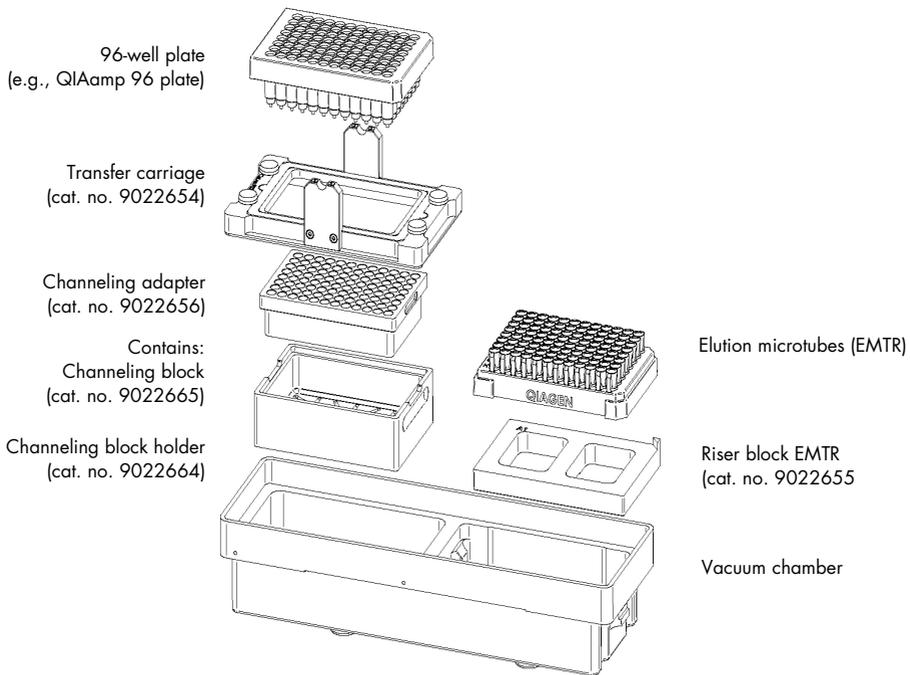


Figure 1. Assembling the vacuum chamber.

Optional features

Processing 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.

Off-board lysis

For some applications, it may be necessary to lyse samples in a safety cabinet. For some sample types, lysis outside the instrument might be necessary. Please refer to Sample Pretreatment Protocols on pages 34–39.

When using an off-board lysis protocol, choose the **QIAamp virus off-board lysis protocol** in the software setup step under the selected protocol.

QIAamp Virus Protocol

This protocol is for the purification of viral nucleic acids from diverse samples. See Table 1 on page 9 for sample pretreatments. There are two types of protocols:

- QIAamp virus protocol: this protocol starts with the lysis of the samples after a necessary pretreatment.
- QIAamp virus off-board lysis protocol: this protocol starts with adding the binding buffer to manually lysed samples.

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, Buffer AW2 and carrier RNA have been prepared according to the instructions in “Preparing reagents and instrument” (page 17).
- When working with difficult samples, use a vacuum performance check to check if all liquid has passed the membrane. See “Troubleshooting Guide” on page 40 and the *QIAcube HT User Manual* for guidance.
- Ensure that the relevant version of the **QIAamp 96 Virus** 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.

- If the volume of the samples is less than 200 µl, add PBS or 0.9% NaCl to a final volume of 200 µl.
- Prepare a mixture of Buffer ACL, carrier RNA and internal control (if applicable) according to Table 2 on page 26.

Manual lysis procedure

Follow this procedure for manual sample lysis if using the QIAamp virus off-board lysis protocol.

1. Add 200 µl of each fluid sample to the bottom of an S-Block well.
2. Add 20 µl of QIAGEN Proteinase K to each sample.
3. Prepare a mixture of Buffer ACL, carrier RNA and internal control (if applicable), according to Table 2 on page 26. Add 160 µl of the Buffer ACL mixture to each sample and mix well.
4. Cover the S-Block with adhesive tape.
5. Incubate for at least 30 min at 56° C.
6. Proceed with “QIAamp virus protocol on the QIAcube HT” on page 23 and select the QIAamp virus off-board lysis protocol.

QIAamp virus 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 Virus** 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. Select the protocol: QIAamp Virus protocol or QIAamp Virus off-board lysis protocol (see section “Off-board lysis” on page 21).

8. 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 28.

9. Click **Next**.

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

11. 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.

12. In the **Plate Assignment** screen, select the samples from the input and assign them to the output plate. Then, click **Next**.

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

It is possible to change the assignment, if required. Select the columns on the output

labware and click **Remove assignment**. Click and hold the left mouse button to mark the columns and rearrange them as needed.

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

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

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

15. 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.

16. Prepare the vacuum chamber as shown in Figure 1 on page 20. 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.

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

Check that the number and position of available and unused tips is the same on the

instrument worktable and in the software workspace.

If more tips are required, you will be prompted to replace empty tip racks with new tip racks during the run. Information about the approximate time for refill will be given in the run screen. For more information, see the *QIAcube HT User Manual*.

18. Transfer the indicated volumes of all reagents into the corresponding reagent troughs.

Close the lids and place the troughs in the indicated positions on the worktable

19. Prepare a mixture of Buffer ACL, carrier RNA and internal control (if applicable), according to Table 2.

Important: Prepare ACL mixture immediately before starting the run.

For the QIAamp Virus protocol place the ACL mixture in the indicated positions on the worktable.

For the QIAamp Virus off-board lysis protocol, you will need the ACL mixture during the manual lysis procedure.

Table 2. Preparation of Buffer ACL, carrier RNA, and internal control (if applicable)

	Number of samples									
	24	32	40	48	56	64	72	80	88	96
Buffer ACL (ml)	4.16	5.44	6.72	8.00	9.28	10.56	11.84	13.12	14.40	15.68
Carrier RNA* (µl)	130	170	210	250	290	330	370	410	450	490
Internal Control* (µl)	130	170	210	250	290	330	370	410	450	490

*If you are not using the Internal Control or Carrier RNA, then use RNase-free water instead.

20. Add the samples to the selected S-Block wells. Place the S-Block in the indicated position of the QIAcube HT worktable.

For the QIAamp Virus protocol, add 200 µl of each sample.

For the QIAamp Virus off-board lysis protocol, add 380 µl of each lysed sample.

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.

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

22. 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*.

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

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

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

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

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

Cleaning the instrument after completing a run

1. Follow the instructions in the QIAcube HT Prep Manager Software for cleaning the instrument after a run.
2. Cover tip racks that contain only unused tips with the lid and remove them from the worktable.
3. Cover fractions of partly used tip racks with an adhesive tape. Then cover the tip racks with the lid and remove from the worktable. Discard empty tip racks.

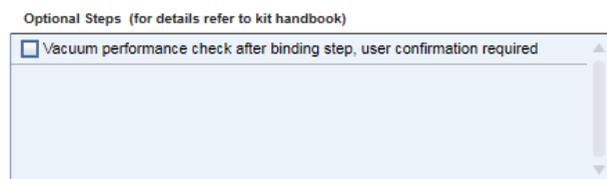
4. If the run has been stopped and the instrument did not remove all used tips, remove them now and discard them.
5. Remove all reagent troughs and discard them.
Note: We recommend not reusing reagents for multiple runs.
6. Remove the input plate.
7. Discard the QIAamp 96 plate or keep partially used QIAamp 96 plates for subsequent reuse. In this case cover used fractions with an adhesive tape.
8. Remove the tip chute and all adapters from the worktable. Remove the carriage, channeling adapter and riser block from the vacuum chamber. Clean all parts as described in the *QIAcube HT User Manual*.
9. Discard the tip disposal box.
10. Clean any reagents that may have spilled on the instrument worktable or vacuum chamber with a damp cloth.
11. Discard all waste according to local safety regulations.

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

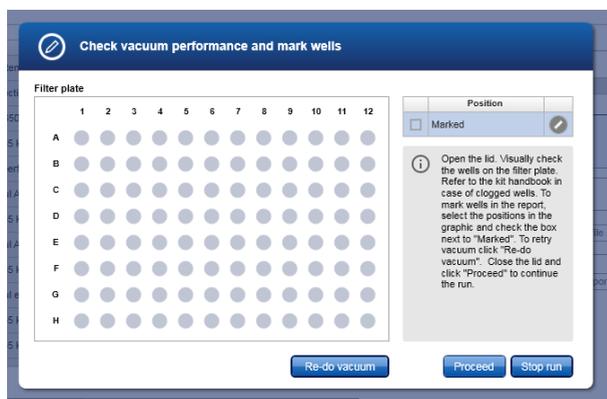
Optional steps

Vacuum performance check

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



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



1. Open the instrument lid.

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

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

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

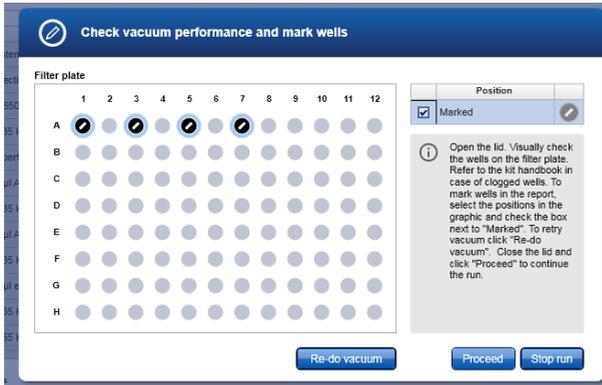
If liquid remains in the wells, click the **Re-do vacuum** button to apply the same vacuum pressure again. The vacuum will be activated for a certain time or until you press the **Stop vacuum** button.

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

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

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

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



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

Advanced options

Important: QIAGEN does not recommend modifying any of the parameters found in the **Advanced options** section.

These parameters have been optimized for each QIAcube HT Kit to guarantee accurate and valid experiment results. QIAGEN is not responsible for the outcome and does not support experiments performed using modified advanced options. Please note that any changes to these options are carried out at your own risk.

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

Advanced options

Vacuum parameter

Vacuum intensity kPa

Vacuum time sec

Elution parameter

Total elution volume μl [90 - 400 μl]

Elution steps ▼

Top elute

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 180 sec for the vacuum time.

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

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

If you want to adapt your protocol you could change the vacuum time from 60 sec to 360 sec. Please note that these changes are not recommended by QIAGEN.

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

Elution parameter

In the **Elution parameter** section, it is possible to change the total elution volume and the elution step.

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

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

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. Please note that these changes are not recommended by QIAGEN.

TopElute

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

By default, the Top Elute option is checked.

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

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

Note: 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.

Sample Pretreatment Protocols

Pretreatment B1 for Respiratory Samples

This protocol is intended for viscous respiratory samples. Non-viscous respiratory samples do not require pretreatment and can be used directly as starting material in “with “QIAamp Virus Protocol”, page 21.

Procedure

1. Liquefy the sample according to either step 1a. or 1b.
 - 1a. Add 1 volume of Sputasol solution to 1 volume of sample, and shake well.
Place in a 37°C water bath, and incubate with periodic shaking until the sample is completely liquefied.
 - 1b. Mix 1 volume of sample with 1 volume of NAC buffer (10 g N-acetylcysteine per liter of 0.9% NaCl solution). If the sample is very viscous or solid (e.g., when working with lower respiratory samples), try to disrupt it mechanically by pipetting up and down. Incubate at room temperature (15–25°C) for 30 min with constant shaking.
For easier pipetting, it may be necessary to cut off the end of the pipette tip.
If the sample is solid, the incubation time needs to be increased to completely liquefy the sample.
2. Centrifuge the liquefied sample to pellet debris, and transfer the clear supernatant to a clean tube.
3. Use 200 µl lysate as starting material and proceed directly with “QIAamp Virus Protocol”, page 21.

Pretreatment B2 for Liquid Transport Media

Most transport media, such as PreservCyt® solution, require no pretreatment and can be used directly as starting material in “QIAamp Virus Protocol”, page 21. For some media such as SurePath® solution, a pretreatment prior to nucleic acid purification can improve extraction efficiency.

Procedure

1. Add 400 µl media to 120 µl Buffer ATL and 30 µl proteinase K solution.
The Buffer ATL and proteinase K solution supplied with the kit might not be sufficient for all users. If necessary, please order separately.
2. Mix the solution carefully.
3. Use 200 µl lysate as starting material and proceed directly with “QIAamp Virus Protocol”, page 21.

Note: Proteinase K can be replaced by Buffer ACL on the QIAcube HT worktable.

Pretreatment B3 for Urine

Procedure

1. Add 400 µl urine to 120 µl Buffer ATL and 30 µl proteinase K solution.
The Buffer ATL and proteinase K solution supplied with the kit might not be sufficient for all users. If necessary, please order separately. See “Ordering Information” on page 46.
2. Mix the solution carefully.
3. Use 200 µl lysate as starting material and proceed directly with “QIAamp Virus Protocol”, page 21.

Note: Proteinase K can be replaced by Buffer ACL on the QIAcube HT worktable.

Pretreatment B4 for Dried Blood Spots

Things to do before starting

- Read “Dried blood spots” on page 14.

Procedure

1. Add dried blot spots (e.g., from Whatman FTA Cards) to 500 µl Buffer ATL and 30 µl proteinase K solution.
The Buffer ATL and proteinase K solution supplied with the kit might not be sufficient for all users. If necessary, please order separately. See “Ordering Information” on page 46.
2. Incubate at 56°C for 1 h with constant agitation.
3. Centrifuge briefly, and transfer 500 µl supernatant to a clean tube.
4. Use 200 µl lysate as starting material and proceed directly with “QIAamp Virus Protocol”, page 21.

Note: Proteinase K can be replaced by Buffer ACL on the QIAcube HT worktable.

Pretreatment B5 for Swabs and Buccal Cells

Things to do before starting

- Read “Swabs” on page 13.

Procedure

1. Add the swab to 800 µl Buffer ATL and 30 µl proteinase K solution.
The Buffer ATL and proteinase K solution supplied with the kit might not be sufficient for all users. If necessary, please order separately. See “Ordering Information” on page 46.
2. Incubate at 56°C for 1 h with constant agitation.
3. Remove the swab from the tube, and centrifuge the tube to collect condensation.

4. Use 200 µl lysate as starting material and proceed directly with “QIAamp Virus Protocol”, page 21.
5. **Note:** Proteinase K can be replaced by Buffer ACL on the QIAcube HT worktable.

Pretreatment F1 for Stool Suspensions

Procedure

1. Mix 1 volume of stool with 9 volumes of 0.9 % NaCl.
2. Vortex vigorously to prepare a homogeneous suspension.
3. Centrifuge the suspension to pellet debris, and transfer the clear supernatant to a clean tube.

Note: Depending on the bacteria species and the speed of centrifugation, bacteria will be present in the pellet.

4. Use 200 µl lysate as starting material and proceed directly with “QIAamp Virus Protocol”, page 21.

Pretreatment T1 Mechanical Disruption of Tissue

This pretreatment is for the extraction of viral RNA and viral DNA from most types of tissue.

Important point before starting

- Stainless steel beads must be ordered separately (see “Ordering Information” on page 46).

Procedure

1. Place up to 5 mg tissue in 2 ml microcentrifuge tubes each containing 1 stainless steel bead (5 mm mean diameter).

Note: Up to 20 mg tissue can be processed. We recommend starting with 5 mg and increasing the amount if needed. For tissues with a very high number of cells for a given

mass of tissue (e.g., spleen), a reduced amount of starting material (5–10 mg) should be used.

If working with fibrous tissue, cutting the tissue into smaller pieces before starting disruption will improve disruption efficiency.

2. Add 300 μ l PBS or 0.9% NaCl solution to each tube.
3. Place the tubes in the TissueLyser II Adapter Set.
4. Operate the TissueLyser II for 2 min at 25 Hz.

Optional: If working with fiber-rich tissue, disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for a further 2 min at 25 Hz.

5. Disassemble the adapter set. Centrifuge the samples at 14,000 \times g for 2 min at room temperature (15–25°C).
6. Use 200 μ l of the supernatant from step 5 as the starting material for “with “QIAamp Virus Protocol”, page 21.
7. For fiber-rich tissues, complete disruption may not always be possible. Ensure that no solid particles are transferred to the purification protocol.

Pretreatment T2 for Enzymatic Digestion of Tissue

This pretreatment is for the extraction of viral DNA from most types of tissue. It is not suitable for viral RNA because the lysis conditions do not sufficiently conserve RNA integrity.

Things to do before starting

- Check Buffer ATL for precipitate upon storage. If it contains precipitates, warm to 56°C to dissolve precipitates.
- Heat a thermomixer block, shaking water bath or rocking platform to 56°C for use in step 3 of the pretreatment protocol.

Procedure

1. Cut up to 5 mg tissue into small pieces and place in a 2 ml microcentrifuge tube. Add 180 μ l Buffer ATL.

Note: Up to 20 mg tissue can be processed. We recommend starting with 5 mg and increasing the amount if needed. For tissues with a very high number of cells for a given mass of tissue (e.g., spleen), a reduced amount of starting material (5–10 mg) should be used.

We recommend cutting the tissue into small pieces for efficient lysis.

2. Add 20 μ l QIAGEN Proteinase K. Close the cap and mix thoroughly by vortexing. Briefly centrifuge the tube to collect any solution from the cap.
3. Incubate at 56°C with constant agitation until the tissue is completely lysed.

Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. If more convenient, overnight lysis is possible but should be evaluated for specific sample types.

After incubation, the lysate may appear viscous, but should not be gelatinous. If a substantial gelatinous pellet remains after incubation and vortexing, extend incubation time at 56°C for the QIAGEN Proteinase K digest and/or increase the amount of QIAGEN Proteinase K to 40 μ l. Reduce the amount of starting material in future preparations of this tissue type.

If no thermomixer, shaking water bath or rocking platform is available, incubate in a heating block or water bath and vortex occasionally during incubation to disperse the sample.

4. Optional: If solid tissue or debris remains in the tubes after lysis, add 50 μ l Buffer ATL. Mix by vortexing and centrifuge at 6,000 \times g (8,000 rpm) for 1 min. Use 200 μ l of the supernatant in the next step.
5. Use 200 μ l lysate as starting material and proceed directly with “QIAamp Virus Protocol”, page 21.

Note: Proteinase K can be replaced by Buffer ACL on the QIAcube HT worktable.

Troubleshooting Guide

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

Comments and suggestions

Instrument issues

- | | |
|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) Recovery in case of 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 not possible to recover the run. Please continue manually. |
| c) Blocked membranes | <p>When processing samples that might potentially block the membrane, we recommend using a vacuum performance check.</p> <p>If liquid is still visible, remove 500 µl using a pipet. Then scrape the surface of the membrane with a fresh pipet tip in order to relocate any solid particles that may block the membrane. Take care not to damage the membrane. If there is still no liquid flow, pipet all liquid from the well and proceed with the run.</p> <p>After the instrument has added wash buffer, open the hood to pause the run. Check if the well is still blocked. If so, remove all liquid using a pipet.</p> <p>Do not perforate the membrane. Uncovered perforated wells will disturb vacuum integrity during elution across the whole plate.</p> <p>Proceed the run. No buffer will float over from the blocked well into other wells from this step on.</p> <p>Next time, use less sample (tissue).</p> |

Comments and suggestions

Little or no viral DNA or RNA 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) 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. |
| c) Insufficient sample lysis | QIAGEN Proteinase K was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh QIAGEN Proteinase K (see storage recommendations on page 5).

For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. In this case, choose the off-board lysis protocol. |
| d) Carrier RNA not added to Buffer ACL mix or degraded carrier RNA | Please refer to the recommendations for preparation, storage and addition of carrier RNA. |
| e) RNase contamination in Buffer AVE | Take care not to introduce RNases, which can degrade viral RNA. This may occur if tubes containing Buffer AVE are opened repeatedly. In case of RNase contamination, replace the open vial of Buffer AVE with a new vial. Repeat the purification procedure with new samples. |
| f) Nucleic acids in samples already degraded prior to purification | Samples were frozen and thawed more than once or stored at room temperature (15–25°C) for too long. Always use fresh samples or samples thawed only once. Repeat the purification protocol with new samples. |

DNA or RNA does not perform well in downstream applications

- | | |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) Little or no DNA or RNA in the eluate | See “Little or no viral DNA or RNA in the eluate”, above. |
| b) Too much eluate in the amplification reaction | Some sample types may contain high amounts of background nucleic acids (e.g. whole blood, tissue) or PCR inhibiting substances (stool). High amounts of background nucleic acids may inhibit amplification reactions, and removal of inhibitors may not be complete without additional treatment. Reduce the amount of sample input and/or the amount of eluate added to the amplification reaction. |
| c) Too much background nucleic acids in the eluate | Determine the maximum amount of carrier RNA suitable for the amplification reaction.

In RT-PCR, a low DNA background is preferable. Use less eluate. |

Comments and suggestions

- d) Performance of purified nucleic acids in assays varies with aging of reconstituted wash buffers
- 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.

Precipitate in buffers

- a) Precipitate in Buffer ACL or Buffer ACB
- Precipitate may form after storage at low temperature or prolonged storage. To dissolve precipitate, incubate Buffer ACL or ACB for 30 min at 37°C with occasional shaking.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Remove RNase contamination from bench surfaces, nondisposable plasticware and laboratory equipment (e.g., pipets and electrophoresis tanks) using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 44), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g.,

* 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.

0.5% SDS),* rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol-resistant), and allow to dry.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent*, thoroughly rinsed and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate), as described in “Solutions” below.

Solutions

Solutions should be purchased RNase-free or treated with 0.1% DEPC. We recommend purchasing RNase-free water. This is because, if trace amounts of DEPC remain after autoclaving buffer, purine residues in RNA might be modified by carbethoxylation and performance of enzymatic reactions such as PCR may be negatively affected. Therefore, residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for at least 15 minutes.

DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the

* 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.

solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris*, buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO₂. When preparing Tris buffers, use RNase-free water to dissolve Tris to make the appropriate buffer.

* 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 Virus QIAcube HT Kit	For 480 preps: QIAamp plates, QIAGEN Proteinase K, Carrier RNA, Buffers	57731
QIAcube HT Plasticware	For 480 preps: 5 S-Blocks, 5 EMTR RS, 2 x 50 Caps for EMTR, 9 x 96 Filter-Tips OnCor C, TapePad	950067
Elution Microtubes RS	24 x 96 Elution Microtubes, racks of 96; includes cap strips	120008
S-Blocks	24 x 96-well blocks with 2.2 ml wells	19585
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
Buffer ASL	4 x 140 ml Stool Lysis Buffer	19082
TissueLyser		
TissueLyser II	Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96*	85300
TissueLyser Adapter Set 2 x 24	2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
TissueLyser LT	Compact bead mill, 100-240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube†	85600

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

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

Product	Contents	Cat. no.
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Pathogen Lysis Tubes L	50 Pathogen Lysis Tubes and 1 vial of Reagent DX	19092
Pathogen Lysis Tubes S	50 Pathogen Lysis Tubes and 1 vial of Reagent DX	19091
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
Accessories		
Collection Microtubes (racked)	(10 x 96) nonsterile polypropylene tubes; 960 in racks of 96	19560
Collection Microtube Caps	For capping collection microtubes and round-well blocks; 960 in strips of 8	19566

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

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

Quick-Start Protocol

This protocol is for use with QIAcube HT Prep Manager software. If you are using QIAcube HT 4.17 software, download the corresponding protocol at www.qiagen.com/HB-1569.

Further information

- *QIAamp 96 Virus QIAcube HT Handbook*: www.qiagen.com/HB-2160
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- This protocol is for the purification of viral nucleic acids from fluid samples using the QIAamp 96 Virus QIAcube HT Kit and QIAcube HT Prep Manager software.
 - See the kit handbook for information on handling various sample types.
 - Prepare Buffers ACB, AW1, AW2 and carrier RNA according to the instructions in the *QIAamp 96 Virus QIAcube HT Handbook*.
 - Prepare a mixture of Buffer ACL, carrier RNA and internal control (if applicable) immediately before starting the run according to Table 1.
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 Virus 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 protocol: QIAamp virus protocol (including lysis) or QIAamp virus off-board lysis protocol (without lysis). 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 for loading the worktable.
8. Add the volume of sample indicated in the **Worktable** step 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 Top Elute fluid, there may be 2 liquid phases in the elution microtubes. Top Elute fluid will be the top layer over the elution buffer.
12. Create a report (if required).
13. Follow the cleaning procedure.

Table 1. Buffer ACL mixture preparation.

Samples	24	32	40	48	56	64	72	80	88	96
Buffer ACL (ml)	4.16	5.44	6.72	8.00	9.28	10.56	11.84	13.12	14.40	15.68
Carrier RNA* (µl)	130	170	210	250	290	330	370	410	450	490
Internal Control* (µl)	130	170	210	250	290	330	370	410	450	490

* If you are not using the IC or Carrier RNA then use RNase-free water instead.

Limited License Agreement for QIAamp 96 Virus QIAcube HT Kit

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

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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