December 2013

cador® Pathogen 96 QIAcube® HT Kit Handbook

For the automated purification of viral RNA and DNA and bacterial DNA from animal whole blood, serum, plasma, other body fluids, swabs and washes, and tissue and feces, using the QIAcube HT or QIAxtractor® instrument

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

***cador* Pathogen 96 QIAcube HT Kit**	**(5)**
Catalog number	**54161**
Number of preps	**480**
QIAamp® 96 plates	5
Buffer VXL*	2 x 30 ml
Buffer ACB*† (concentrate)	2 x 60 ml
QIAGEN Proteinase K	2 x 6 ml
Carrier RNA (poly A)	2 x 310 µg
Buffer AW1*‡ (concentrate)	2 x 98 ml
Buffer AW2‡ (concentrate)	2 x 66 ml
Buffer AVE§	2 x 125 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 7 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 number	**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 –20°C. Do not subject aliquots of carrier RNA to more than 3 freeze–thaw cycles. Carrier RNA dissolved in Buffer AVE and Buffer VXL is stable at room temperature (15–25°C) for up to 48 hours (see "Preparing reagents" on page 16 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 *cador* Pathogen 96 QIAcube HT Kit is intended for the automated extraction of pathogen nucleic acids (viral RNA and DNA and bacterial DNA) from animal whole blood, serum, plasma, swabs, washes, tissue, and feces using the QIAcube HT instrument. For laboratory use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a veterinary 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, 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 *cador* Pathogen 96 QIAcube HT Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *cador* Pathogen 96 QIAcube HT Kit enables the efficient purification of viral RNA and DNA and bacterial DNA from a broad range of animal samples including whole blood, serum, plasma, swabs, washes, tissue, and feces (see "Starting material" on page 13). The extracted nucleic acids are free of proteins, nucleases, and other impurities, and are ready for use in downstream applications, such as real-time PCR-based pathogen identification.

For maximum convenience, this kit uses the same principles and reagents as the QIAamp *cador* pathogen Mini Kit.

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 VXL, 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 pathogen species and must be validated by the user. Some samples may require a pretreatment (see page 9).

Description of protocols

There are two types of protocol in this handbook. Samples will either directly undergo nucleic acid purification, or undergo pretreatment followed by nucleic acid purification.

Most sample types can be directly processed without pretreatment. However, depending on the starting material and the target pathogen, one of the pretreatment protocols may be needed. Table 1 on page 9 provides an overview of which pretreatment protocols are suited to which starting material and pathogen combinations.

- Nucleic acid purification protocol (page 22)
- Pretreatment protocols (pages 28–34)

Table 1. Pretreatment protocols for fluid and tissue samples

Sample	Target	Pretreatment	Page
Fluids e.g., whole blood, serum, plasma, swab or wash fluid, pretreated tissue	Viral RNA and DNA, DNA of easy-to-lyse bacteria*	–	22
Whole blood or pretreated tissue	DNA of difficult-to-lyse bacteria*	**Pretreatment B1** For difficult-to-lyse bacteria in whole blood or pretreated tissue	28
Serum, plasma, swabs, washes, body cavity fluids, urine	DNA of difficult-to-lyse bacteria*	**Pretreatment B2** For difficult-to-lyse bacteria in body fluids†	29
Tissue e.g., liver, spleen, kidney, lymph node	Viral RNA and DNA‡	**Pretreatment T1** Mechanical disruption of tissue	30
	Viral DNA§, bacterial DNA¶	**Pretreatment T2** Enzymatic digestion of tissue	31
Feces	Viral RNA and DNA‡	**Pretreatment F1** Non-lysing suspension method	33
	Bacterial DNA* and viral DNA	**Pretreatment F2** Lysing suspension method	34

* Gram-positive bacteria are difficult to lyse due to their rigid cell wall. Many Gram-negative bacteria are easy to lyse, but some are difficult to lyse and will also benefit from Pretreatment B1 or B2.

† Not suitable for whole blood.

‡ Not suitable for bacterial DNA due to centrifugation step (see page 33).

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

¶ For difficult-to-lyse bacteria, subsequently use Pretreatment B1 (page 28).

For further pretreatment recommendations, contact QIAGEN Technical Services.

Nucleic acid purification protocol

The protocol "Purification of Pathogen Nucleic acids from Fluid Samples" (page 22) is optimized for purification of viral RNA and DNA and the DNA of easy-to-lyse bacteria from up to 200 µl of fluid material. Suitable starting materials for direct processing using this method include:

- Whole blood
- Serum
- Plasma
- Body cavity fluids (e.g., peritoneal, synovial, cerebrospinal)
- Liquid extracts from swabs (e.g., nasal, pharyngeal, and cloacal swabs)
- Wash fluids (e.g., from bronchoalveolar lavages)
- Other fluids, such as urine or feces suspensions*

Pretreatments

The various pretreatments included in this handbook are optimized for specific combinations of starting material and target pathogen. 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 page 11)

* The processing of samples with high inhibitor content, such as urine and feces, may require a reduction in sample input volume or further measurements. A list is available from QIAGEN Technical Services.

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 Software version 4.17.1 or higher
- Reagent troughs
- Vortexer

Pretreatment B1 — for difficult-to-lyse bacteria in whole blood or pretreated tissue

- Vortexer with Microtube Foam Insert (Scientific Industries, cat. no. 504-0234-00) or TurboMix Attachment (Scientific Industries, cat. no. SI-0564); or FastPrep®-24 (MP Biomedicals, cat. no. 6004500), or TissueLyser II (cat. No. 85300) with a TissueLyser II Adapter Set 2 x 24 (QIAGEN, cat. no. 69982) or 2 x 96 (QIAGEN, cat. no. 69984), or TissueLyser LT (QIAGEN, cat. no. 85600) with the TissueLyser LT Adapter for 12 tubes (QIAGEN, cat. no. 69980)‡
- Pathogen Lysis Tubes L (QIAGEN, cat. no. 19092) containing 50 Pathogen Lysis Tubes with glass beads and 1 vial Reagent DX (QIAGEN, cat. no. 19088) for bead-beating of bacteria
- Buffer ATL (QIAGEN, cat. no. 19076)

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

† To process dedicated QIAcube HT kits on the QIAxtractor instrument, QIAcube HT Software version 4.17.1 or higher is needed together with the Accessories Pack, QXT see ordering information, page 40).

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

Pretreatment B2 — for difficult-to-lyse bacteria in body fluids

- Vortexer with Microtube Foam Insert (Scientific Industries, cat. no. 504-0234-00) or TurboMix Attachment (Scientific Industries, cat. no. SI-0564); or FastPrep-24 (MP Biomedicals, cat. no. 6004500), or TissueLyser II (QIAGEN, cat. no. 85300) with a TissueLyser II Adapter Set 2 x 24 (QIAGEN, cat. no o. 69982) or 2 x 96 (QIAGEN, cat. no. 69984), or TissueLyser LT (QIAGEN, cat. no. 85600) with the TissueLyser LT Adapter for 12 tubes (QIAGEN, cat. no. 69980)*
- Pathogen Lysis Tubes L (QIAGEN, cat. no. 19092) or S (QIAGEN, cat. no. 19091) containing 50 Pathogen Lysis Tubes with glass beads and 1 vial Reagent DX (QIAGEN, cat. no. 19088) for bead-beating of bacteria
- Buffer ATL (QIAGEN, cat. no. 19076)

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*

  Note: A vortexer with Microtube Foam Insert (Scientific Industries, QIAGEN, cat. no. 504-0234-00) can also be used
- 5 mm stainless steel beads (QIAGEN, cat. no. 69989)
- PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl) or NaCl 0.9%

Pretreatment T2 — enzymatic digestion of tissue

- Thermoshaker suitable for 2 ml collection tubes
- Buffer ATL (QIAGEN, cat. no. 19076)

Pretreatment F1 — isolation of viral nucleic acids from feces

- Distilled water or RNase-Free Water (QIAGEN, cat. no. 129112)

Pretreatment F2 — isolation of bacterial and viral DNA from feces

- Buffer ASL (QIAGEN, cat. no. 19082)

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

Important Notes

Starting material

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 user confirmation step to check if all liquid has passed the membrane. See "Troubleshooting guide" and the QIAcube HT manual for guidance.

Highly viscous fluids may require a treatment to reduce their viscosity to allow for efficient extraction of pathogen nucleic acids. Please contact QIAGEN Technical Services for recommendations.

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

Animal whole blood

Blood samples treated with EDTA, citrate, or heparin 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 –20°C or –80°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.

For blood samples containing nucleated erythrocytes (e.g., samples from bird and fish), use 5–25 µl blood, and adjust the sample volume to 200 µl with PBS or 0.9% NaCl.

Animal serum, plasma, other body fluids, swab, and wash specimens

Frozen plasma or serum must not be thawed more than once before processing.

We recommend storing swabs in transport media; for example, viral transport media (VTM) or brain–heart infusion broth (BHI). Remove the swab and squeeze out the liquid by pressing the swab against the inside of the storage tube. For extraction of viral RNA or DNA, we recommend centrifuging the swab media briefly to ensure any residual solid materials are removed.

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

Up to 200 µl serum, plasma, other body fluid, swab media supernatant, or wash fluid 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 15 for information about the use of carrier RNA).

The processing of samples with very high inhibitor contents, such as urine or fecal suspensions, 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.

For extraction of bacterial DNA, the input volume can be increased to more than 200 µl, for example, 1.5 ml for increased sensitivity of bacterial detection. Gram-negative bacteria in cell-free fluids can be concentrated by centrifugation of higher volumes. Resuspend pellets in PBS and use 200 µl as starting volume. See Pretreatment B2 (page 29) for extraction of DNA from difficult-to-lyse bacteria.

Animal 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 pathogen nucleic acids in the tissue may vary depending on tissue type, pathogen, and stage of infection. Additional pretreatments for tissue samples are available at QIAGEN technical service, including a rapid protocol and recommendations for difficult tissues.

Up to 25 mg of fresh or frozen tissue can be used as a starting amount. For tissues with a very high number of cells for a given mass of tissue, such as spleen, a reduced amount of starting material (5–10 mg) should be used.

Using too much input material decreases the quality and the amount of DNA and leads to an increased risk of blocked membranes.

Yields of nucleic acids

For samples containing a low amount of cells (e.g., serum), the yield of viral or bacterial 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 *cador* Pathogen 96 QIAcube HT protocol recovers total nucleic acids. Therefore, cellular DNA and RNA will be co-purified from any cells in the sample along with viral RNA and DNA and bacterial DNA 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 pathogen nucleic acid yields.

Using carrier RNA and internal controls

Carrier RNA

We recommend adding carrier RNA to fluids containing a low amount of cells such as serum, plasma, swab media, and wash fluid. This enhances adsorption of viral RNA and DNA and bacterial 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 pathogen nucleic acids.

Internal control

Use of an internal control, such as the QIAGEN Internal Control (to be used with QuantiFast® Pathogen +IC Kits, see page 40 for ordering information), is optional, depending on the amplification system used. Using the *cador* Pathogen 96 QIAcube HT Kit in combination with amplification systems that use an internal control may require introduction of these internal controls during the purification procedure to monitor the efficiency of sample preparation and downstream assay.

Add unprotected internal control nucleic acids (e.g., plasmid DNA or in-vitro transcribed RNA) to VXL mixture only. Do not add these internal control nucleic acids directly to the sample.

The amount of internal control added depends on the assay system and the elution volume. Evaluation of the correct amount of internal control nucleic acid must be performed by the user. Refer to the manufacturer's instructions to determine the optimal concentration of internal control. For the standard protocol, it is recommended to add 5 µl internal control solution. When using Internal Control DNA (High conc.) (QIAGEN, cat. no. 211392), simply resuspend the lyophilizate with 550 µl QuantiTect® Nucleic Acid dilution buffer instead of 1100 µl.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing the purified viral RNA and DNA or bacterial DNA at 2–8°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at –20°C, or even –80°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. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Preparing reagents

Carrier RNA stock solution

For use, lyophilized carrier RNA should first be dissolved in Buffer AVE. Add 310 µl Buffer AVE to the tube containing 310 µg lyophilized carrier RNA to obtain a stock solution of 1 µg/µl. Add this solution to Buffer VXL as described below. Unused carrier RNA dissolved in Buffer AVE should be frozen in aliquots at –20°C. Aliquots of carrier RNA should not be subjected to more than 3 freeze–thaw cycles.

QIAGEN Proteinase K

The *cador* Pathogen 96 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.

Add QIAGEN Proteinase K to Buffer VXL immediately before starting the protocol.

Buffer ACB

Buffer ACB is supplied as a concentrate. Before using for the first time, 40 ml isopropanol (100%) must be added, 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, 130 ml ethanol (96–100%) must be added to each bottle of Buffer AW1, 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, 160 ml ethanol (96–100%) must be added, 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.

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 will 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.

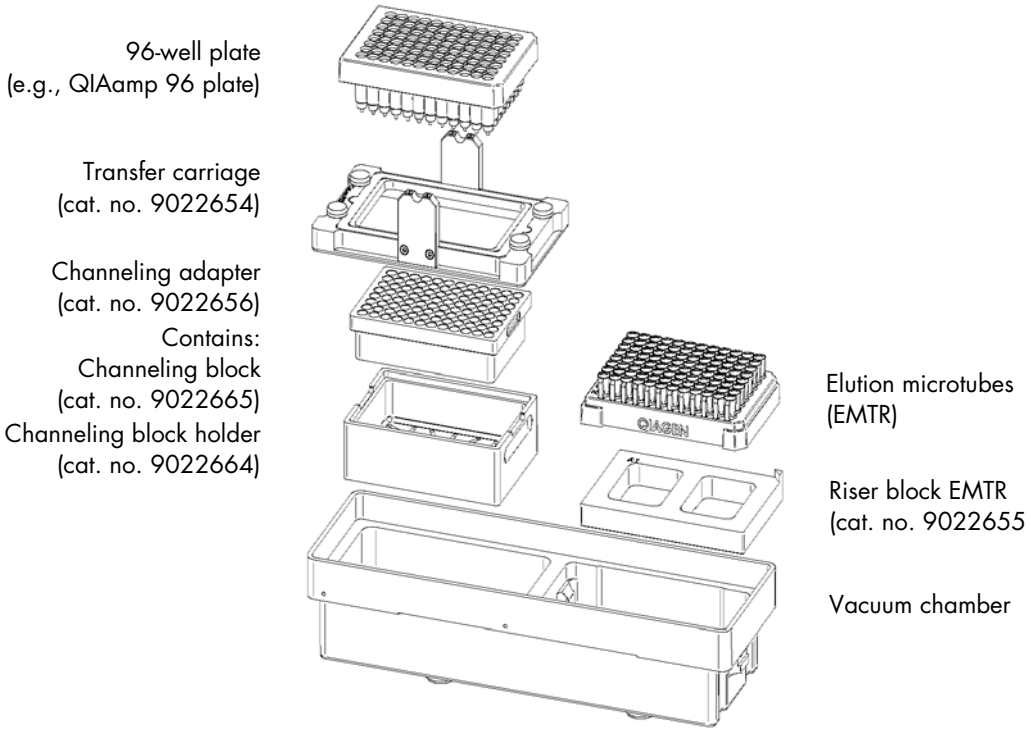


Figure 1. Assembling the vacuum chamber.

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 40.

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.

Off-board lysis

For some applications, it may be necessary to lyse samples in a safety cabinet. For some sample types a heated lysis outside the instrument might enhance performance. A protocol allowing for lysis outside the instrument is available from QIAGEN Technical Services.

Integrated DNase digestion

Some sample types may contain high amounts of background nucleic acids (e.g., animal whole blood, tissue). High amounts of background nucleic acids may inhibit amplification reactions, especially reverse transcription. An additional protocol reducing the amount of DNA by DNase digestion on the membrane is available from QIAGEN Technical Services.

Amount of Elution Buffer

The default volume of Buffer AVE per well is 150 µl. This amount was determined to work best with the variety of samples the kit is able to process. When using a more narrow set of samples, this amount might be varied from 100 to 200µl under close monitoring of performance.

Sample data input, data tracking, and LIMS connection

In the software environment information about an item could 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 ask QIAGEN Technical Service for a detailed example.

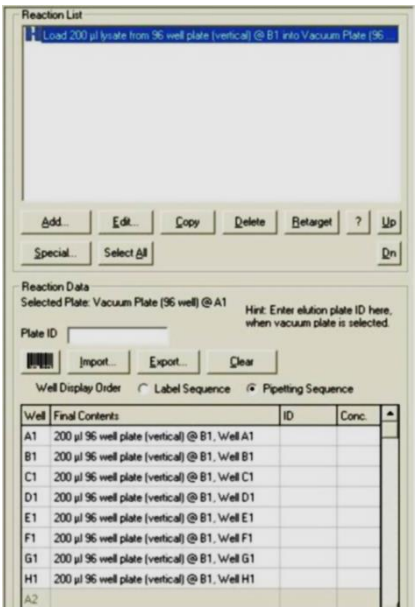


Figure 2: Example for right-hand pane information.

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 Pathogen Nucleic Acids from Fluid Samples

This protocol is for the purification of viral RNA and DNA and the DNA of easy-to-lyse bacteria from fluid samples or pretreated tissue samples.

Important points before starting

- Even though lysis at room temperature is very effective, lysis of some pathogens might benefit from a 10 minute lysis step at 70°C in an external heater. Protocols are available at QIAGEN Technical Service
- 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, and carrier RNA have been prepared according to the instructions in "Preparing reagents" (page 16).
- When working with difficult samples, use a user confirmation step to check if all liquid has passed the membrane. See "Troubleshooting guide" and the QIAcube HT manual for guidance.
- Ensure that the relevant version of the *cador* Pathogen 96 QIAcube HT .QSP run file is installed on the instrument.

  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.**

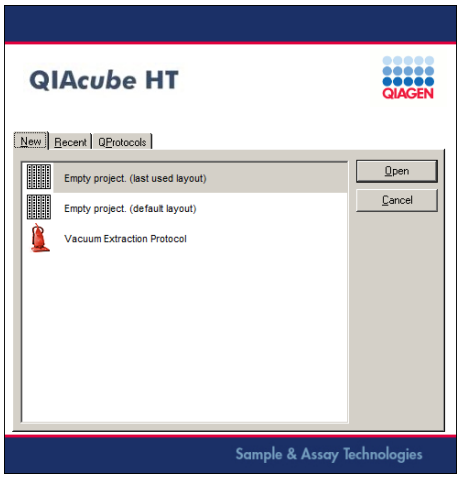
   Ensure that the tip discard chute is open and unblocked. Remove the UV protective cap from the tip chute.

   Ensure 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 at least all steps until and including lysate transfer, 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 workspace, 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 18. See the *QIAcube HT User Manual* for more information.**

    Note: 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: 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.

14. **Add 200 µl 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 per columns samples 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.

15. **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.**

16. **Prepare the indicated volume of VXL mixture according to table below and mix well.**

Table 2. Preparation of Buffer VXL, carrier RNA, internal control (if applicable) and QIAGEN Proteinase K mixture.

Samples	8	16	24	32	40	48
Buffer VXL (ml)	1.4	2.1	2.7	3.4	4.0	4.6
QIAGEN Proteinase K (µl)	360	520	680	840	1000	1160
Carrier RNA (µl)	18	26	34	42	50	58
Internal Control (µl)	90	130	170	210	250	290
Samples	56	64	72	80	88	96
Buffer VXL (ml)	5.3	5.9	6.6	7.2	7.8	8.5
QIAGEN Proteinase K (µl)	1320	1480	1640	1800	1960	2120
Carrier RNA (µl)	66	74	82	90	98	106
Internal Control (µl)	330	370	410	450	490	530

IMPORTANT: Prepare VXL mixture immediately before starting the run.

17. **Start the run immediately by clicking**  **.**

    The pre-run check appears.

18. **Perform the pre-run check.**

Check the state of the worktable items.

Confirm that worktable is setup 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 check 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 side 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 chamber, when the protocol is complete.**

    See the *QIAcube HT User Manual* for detailed instructions.

    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.

Cleaning the instrument after completing a run

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

   Note: We recommend not reusing reagents in multiple runs. Reagents provided are sufficient for at least 10 runs of 48 samples.

   Note: Do not clean the trough containing TopElute Fluid with water, but 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.**

   Note: 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.

Pretreatment B1 — for Difficult-to-Lyse Bacteria in Whole Blood or Pretreated Tissue

This pretreatment is for the extraction of DNA of difficult-to-lyse bacteria from whole blood or pretreated tissue.

Important points before starting

- Buffer ATL and Pathogen Lysis Tubes L (including Reagent DX) must be ordered separately (for ordering information, see page 40).
- Check Buffer ATL for precipitate upon storage. If it contains precipitates, warm to 56°C to dissolve precipitates.

Things to do before starting

- Add 100 µl Reagent DX to 15 ml Buffer ATL. If smaller amounts are needed, transfer 1.5 ml of Buffer ATL into a sterile 2 ml vial and add 10 µl Reagent DX. Mix well after addition of Reagent DX. After preparation, the mixture is stable for 6 months at room temperature (15–25°C).

Procedure

1. **Add 100 µl Buffer ATL (containing Reagent DX) into a fresh Pathogen Lysis Tube.**
2. **Add 400 µl blood or other sample fluids.**

   If using less starting material, adjust the volume to 400 µl with PBS or 0.9% NaCl.
3. **Place the Pathogen Lysis Tube on a vortexer with a Microtube Foam Insert and vortex for 10 min at maximum speed.**

   Alternatively, the Pathogen Lysis Tube may be processed on a TissueLyser LT for 10 min at 50 Hz, or on a FastPrep-24 by applying a velocity of 6.5 m/s for two 45 s periods with a 5 min resting time between them.
4. **Remove the Pathogen Lysis Tube from the vortexer and briefly centrifuge the tube to remove drops from the inside of the lid.**

   Use 200 µl of the supernatant as starting material for the protocol "Purification of Pathogen Nucleic acids from Fluid Samples" on page 22.

Pretreatment B2 — for Difficult-to-Lyse Bacteria in Body Fluids

This pretreatment is for the extraction of bacterial DNA of difficult-to-lyse bacteria from cell-free fluids, such as serum.

Important points before starting

- Buffer ATL and Pathogen Lysis Tubes L or S (including Reagent DX) must be ordered separately (for ordering information, see page 40).

  Note: Evaluate whether L or S tubes are suitable depending on the bacterial target.

- Check Buffer ATL for precipitate upon storage. If it contains precipitates, warm to 56°C to dissolve precipitates.

Things to do before starting

- Add 100 µl Reagent DX to 15 ml Buffer ATL. If smaller amounts are needed, transfer 1.5 ml of Buffer ATL into a sterile 2 ml vial and add 10 µl Reagent DX. Mix well after addition of Reagent DX. After preparation, the mixture is stable for 6 months at room temperature (15–25°C).

Procedure

1. **Add up to 1.5 ml fluid sample to the Pathogen Lysis tube and centrifuge the tube for 5 min at maximum speed (>14,000 x *g*).**
2. **Remove and discard the supernatant. Repeat steps 1 and 2, if necessary.**
   Use a pipet to remove the supernatant.
3. **Add 500 µl Buffer ATL (containing Reagent DX) and resuspend the pellet.**
4. **Place the Pathogen Lysis Tube on a vortexer with a Microtube Foam Insert and vortex for 10 min at maximum speed.**
   Alternatively, the Pathogen Lysis Tube may be processed on a TissueLyser LT for 10 min at 50 Hz, or on a FastPrep-24 by applying a velocity of 6.5 m/s for two 45 s periods with a 5 min resting time between them.
5. **Remove the Pathogen Lysis Tube from the vortexer and briefly centrifuge the tube to remove drops from the inside of the lid.**
   Use 200 µl of the supernatant as starting material for the protocol "Purification of Pathogen Nucleic acids from Fluid Samples", on page 22.

Pretreatment T1 — Mechanical Disruption of Tissue

This pretreatment is for the extraction of viral RNA and viral DNA from most types of tissue. It is not suitable for bacterial DNA due to the centrifugation step.

Important point before starting

- Stainless steel beads must be ordered separately (for ordering information, see page 40).

Procedure

1. **Place up to 25 mg tissue in 2 ml microcentrifuge tubes each containing 1 stainless steel bead (5 mm mean diameter).**

   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 µl 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: Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outer most, and reassemble the adapter set, if working with fiber-rich tissue. Operate the TissueLyser II for a further 2 min at 25 Hz.

5. **Disassemble the adapter set. Centrifuge the samples at 14,000 x *g* for 2 min at room temperature (15–25°C).**
6. **Use 200 µl of the supernatant from step 5 as the starting material for the protocol "Purification of Pathogen Nucleic acids from Fluid Samples", page 22.**

   For fiber-rich tissues, complete disruption may not always be possible. Ensure that no solid particles are transferred to the purification protocol.

Pretreatment T2 — Enzymatic Digestion of Tissue

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

Important point before starting

- Buffer ATL must be ordered separately (for ordering information, see page 40).

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 25 mg tissue into small pieces and place in a 2 ml microcentrifuge tube. Add 180 µl Buffer ATL.**

   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 µl QIAGEN Proteinase K. Close the cap and mix thoroughly by vortexing. Briefly centrifuge the tube to collect any solution from the cap.**

   Note: When samples pretreated as described above are used in the subsequent nucleic acid purification protocol ("Purification of Pathogen Nucleic acids from Fluid Samples" on page 22), carrier RNA and QIAGEN Proteinase K can be omitted from the VXL mixture (Table 2, page 25). In this case, substitute the volumes of carrier RNA and QIAGEN Proteinase K with PBS or water.

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 QIAGEN Proteinase K digest

and/or increase amount of QIAGEN Proteinase K to 40 µl. 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 for viral DNA or DNA of easy-to-lyse bacteria; Not suitable for difficult-to-lyse bacteria: If solid tissue or debris remains in the tubes after lysis, add 50 µl Buffer ATL. Mix by vortexing and centrifuge at 6,000 x *g* (8,000 rpm) for 1 min. Use 200 µl of the supernatant in step 5.**
5. **Use 200 µl lysate as starting material for step 5a or 5b.**

   IMPORTANT: Ensure that no solid particles are transferred to the next protocol.

5a. For isolation of viral DNA or DNA from easy-to-lyse bacteria, proceed directly with the protocol "Purification of Pathogen Nucleic Acids from Fluid Samples" (page 22).

Note: Do not use QIAGEN Proteinase K in step 1 of the purification protocol.

5b. For isolation of DNA from difficult-to-lyse bacteria, proceed with Pretreatment B1 (page 28).

Pretreatment F1 — for Isolation of Viral Nucleic Acids from Feces

This pretreatment is for extraction of viral RNA and DNA from fecal samples.

Important point before starting

- This pretreatment is not suitable for isolation of bacterial DNA.

Procedure

1. **Add up to 100 mg fecal material to a 2 ml microcentrifuge tube.**
2. **Add 1 ml water and suspend fecal material by vigorous vortexing.**
3. **Centrifuge for 1 min at maximum speed (>14,000 x *g*).**
4. **Use up to 200 µl supernatant as starting material for the protocol, "Purification of Pathogen Nucleic Acids from Fluid Samples" (page 22).**
   Ensure that no solid particles are transferred to the purification protocol.
   Adjust to 200 µl with water, if using a lower volume.

Pretreatment F2 — for Isolation of Bacterial and Viral DNA from Feces

Note: Due to the high diversity of fecal samples, QIAGEN has developed a variety of pretreatments for *cador* Pathogen kits. If any issues regarding inhibition or sensitivity (e.g. difficult-to-lyse bacteria) are encountered, contact QIAGEN Technical Services for advice.

Important point before starting

- Buffer ASL must be ordered separately (for ordering information, see page 40).

Procedure

1. **Add up to 100 mg fecal material to a 2 ml microcentrifuge tube.**
2. **Add 1 ml Buffer ASL and suspend fecal material by vigorous vortexing.**
3. **Incubate suspension for 5 minutes at 70°C.**
   Note: An additional incubation on ice for 5 min may facilitate precipitation of additional inhibitors.
4. **Centrifuge at 14,000 x *g* for 2 min.**
5. **Use up to 200 µl supernatant as starting material for the protocol, "Purification of Pathogen Nucleic Acids from Fluid Samples (page 22).**
   Ensure that no solid particles are transferred to the purification protocol.
   If using a lower volume, adjust to 200 µl with PBS or water.

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, see back cover or 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 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.

	Comments and suggestions
c) Blocked membranes	When processing samples that might potentially block the membrane, we recommend using a user confirmation step after addition of the first wash buffer (AW1). 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. After the instrument has added Buffer AW2, open the hood to pause the run. Check if the well is still blocked. If so, remove all liquid using a pipet and mark well as invalid. Do not perforate the membrane. Uncovered perforated wells will disturb vacuum integrity during elution across the whole plate. Proceed the run. No buffer will float over from the blocked well into other wells from this step on. Next time, use less sample (tissue) and prolong the digestion step.

Little or no pathogen 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.

	Comments and suggestions
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 6). For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. For this purpose an off-board-lysis protocol is available.
d) Carrier RNA not added to Buffer VXL or degraded carrier RNA	Please refer to the recommendations for preparation, storage, and addition of carrier RNA.
e) Buffer VXL- Proteinase K-carrier RNA mixture mixed insufficiently	Mix well by pipetting with a large pipet.
f) 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.
g) 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 pathogen DNA or RNA in the eluate", above.

	Comments and suggestions
b) Too much eluate in the amplification reaction	Some sample types may contain high amounts of background nucleic acids (e.g. animal whole blood, tissue) or PCR inhibiting substances (feces). 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 or use DNase during nucleic acid extraction. An additional protocol is available from QIAGEN Technical Services.
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.
e) Residual ethanol in the eluate	Perform the drying step at 25 kPa for 5 minutes.
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, e.g. after enzymatic digestion of tissue, precipitate may form after addition of Buffer VXL to the sample (step 3 of the protocol "Purification of Pathogen Nucleic acids from Fluid Samples", page 22). The precipitate does not influence subsequent protocol steps and can be dissolved by brief incubation at 56°C.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
cador Pathogen 96 QIAcube HT Kit	For 480 preps: QIAamp 96 plates, QIAGEN Proteinase K, Carrier RNA, Buffers	54161
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
QIAamp *cador* Pathogen Mini Kit (50)	For 50 preps: QIAamp Mini Columns, QIAGEN Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	54104
QIAamp *cador* Pathogen Mini Kit (250)	For 250 preps: QIAamp Mini Columns, QIAGEN Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	54106
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 (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

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

Product	Contents	Cat. no.
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
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
Internal Control RNA (High conc.)	For approximately 100 sample preps (depending on elution volume): Lyophilized Internal Control RNA, Nucleic Acid Dilution Buffer	211492
Internal Control DNA (High Conc.)	For approximately 100 sample preps (depending on elution volume): Lyophilized Internal Control DNA, Nucleic Acid Dilution Buffer	211392
QuantiFast Pathogen RT-PCR +IC Kit (100)*	For 100 x 25 µl reactions: Master Mix, RT Mix, lyophilized Internal Control Assay, lyophilized Internal Control RNA, ROX™ Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE	211452

* Larger Kit sizes available; please inquire.

Product	Contents	Cat. no.
QuantiFast Pathogen PCR +IC Kit (100)*	For 100 x 25 µl reactions: Master Mix, lyophilized Internal Control Assay, lyophilized Internal Control DNA, ROX Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE	211352
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

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.

* Larger Kit sizes available; please inquire.

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