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cador® Pathogen 96 QIAcube® HT Handbook

For automated purification of viral RNA and DNA and bacterial DNA from animal blood, serum, plasma, body fluids, oral fluids, swabs, washes, tissue and feces using QIAcube HT and QIAcube HT Prep Manager Software

Sample to Insight

QIAGEN

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Kit Contents

***cador* Pathogen 96 QIAcube HT Kit**	**(5)**
Catalog no.	**54161**
Number of preps	**480**
QIAamp 96 plates	5
Buffer VXL*	3 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 Safety Information on page 6.

† 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 and Buffer VXL 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 *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, oral fluids, swabs, washes, tissue, and feces using the QIAcube HT instrument. The *cador* Pathogen 96 QIAcube HT Kit is intended for laboratory use. This product is not intended 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.

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, oral fluids, 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. 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 and coated with Ethanol. 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 8).

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 sample starting material and the target pathogen, 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:

- *cador* Pathogen Protocol (page 22)
- Sample Pretreatment Protocols (pages 34–38)

Nucleic acid purification protocol

- The protocol "*cador* Pathogen Protocol" (page 22) is optimized for purification of viral RNA and DNA and DNA from easy-to-lyse bacteria from fluid samples or pretreated tissue samples. Suitable starting materials for direct processing using this method include:
- Cell-free body fluids such as plasma, serum and swab and wash fluids
- Whole blood

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 8 summarizes the pretreatments and their applications.

Some of the pretreatments may require additional components (see "Equipment and Reagents to Be Supplied by User" on page 10).

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*	Not required	–
Whole blood or pretreated tissue	DNA of difficult-to-lyse bacteria*	Pretreatment B1 for difficult-to-lyse bacteria in whole blood or pretreated tissue	34
Serum, plasma, swabs, washes, body cavity fluids, urine	DNA of difficult-to-lyse bacteria*	Pretreatment B2 for difficult-to-lyse bacteria in body fluids†	35
Oral fluids		Pretreatment O1 for oral fluids	36
Tissue e.g., liver, spleen, kidney, lymph node	Viral RNA and DNA‡	Pretreatment T1 mechanical disruption of tissue	36
Tissue e.g., liver, spleen, kidney, lymph node	Viral DNA§, bacterial DNA¶	Pretreatment T2 for enzymatic digestion of tissue	37
Feces	Viral RNA and DNA‡	Pretreatment F1 for Isolation of viral nucleic acids from feces	39
Feces	Bacterial DNA* and viral DNA	Pretreatment F2 for isolation of bacterial and viral DNA from feces	40

* 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 39).

§ 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 34).

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 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 (aScientific 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)†

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

- 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 (cat. no. 19076)

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, 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)

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 as this can reduce flow through the membrane (e.g., blood clots, solid tissue, swab fibers, etc.). When working with difficult samples, use a vacuum performance check to check if all liquid has passed the membrane. See "Troubleshooting Guide" on page 41 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.

Animal whole blood

Blood samples treated with anticoagulants such as EDTA or citrate 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.

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, swabs, oral fluids 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 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 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 35) 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.

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 low amounts 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 44 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.

Internal control RNA or DNA should be added together with the carrier RNA to the lysis buffer (see Table 2 on page 27). Refer to the assay manufacturer's instructions in order to determine the optimal concentration. 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.

When using Internal Control DNA (High conc.) (QIAGEN, cat. no. 211392), simply resuspend the lyophilized material 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. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Preparing reagents and instrument

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 in Table 2 on page 27. 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 *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, 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.

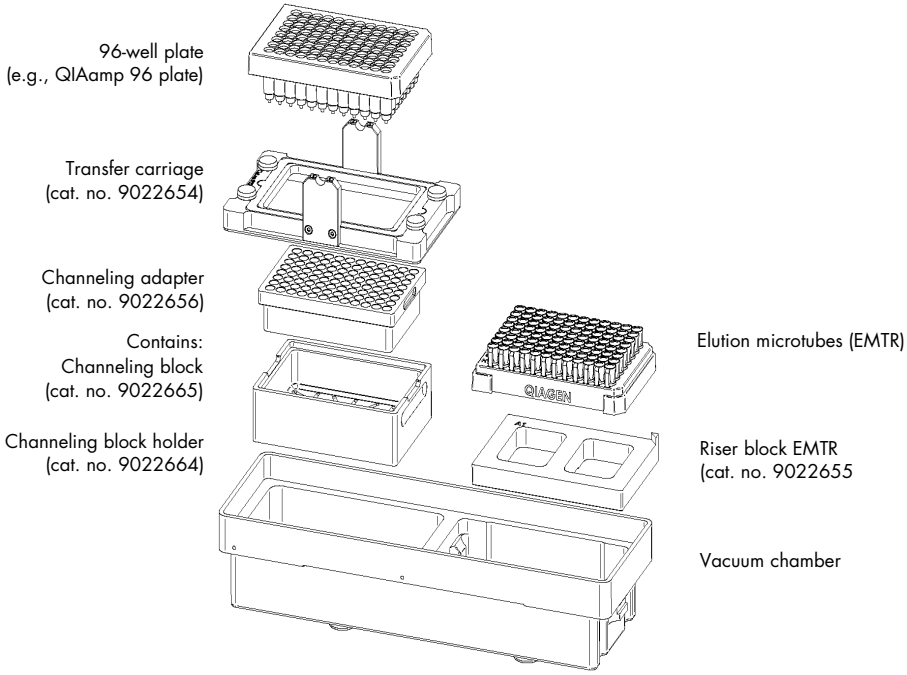


Figure 1. Assembling the vacuum chamber.

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, a heated lysis outside the instrument might enhance performance. Please refer to Sample Pretreatment Protocols on pages 34–38. If lysis with Proteinase K is carried out off-board, Proteinase K may be exchanged with Buffer ACL when setting up the worktable.

When using an off-board lysis protocol, choose the ***cador* pathogen heated off-board lysis protocol** in the software setup step under the selected protocol.

cador Pathogen Protocol

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. There are two types of protocols:

- *cador* pathogen protocol: this protocol starts with the lysis of the samples after a necessary pretreatment.
- *cador* pathogen heated off-board lysis protocol: this protocol starts with adding the binding buffer to manually lysed samples.

Important points before starting

- Do not overload the QIAamp membrane as this can lead to impaired nucleic acid extraction and /or performance in downstream assays
- Avoid repeating freezing and thawing of samples as this may reduce nucleic acid yield and quality

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 41 and the *QIAcube HT User Manual* for guidance.
- Ensure that the relevant version of the ***cador* pathogen** 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.

Manual lysis procedure

Follow this procedure for manual sample lysis if using the *cador* pathogen heated off-board lysis protocol.

1. Add 200 µl of each fluid sample to the bottom of an S-Block well.
2. Prepare a mixture of Buffer VXL, proteinase K, carrier RNA and internal control (if applicable), according to Table 2 on page 27. Add 180 µl of the Buffer VXL mixture to each sample in the S-Block.
3. Cover the S-Block with adhesive tape.
4. Incubate at 70°C, with constant agitation, for 10–15 min.
5. Optional: Briefly centrifuge the S-block to remove liquid from the inside of the tape.
6. Remove the adhesive tape from the S-Block.
7. Proceed with the "*cador* pathogen protocol on the QIAcube HT" on page 23 and select the heated off-board lysis protocol.

cador pathogen 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 ***cador* pathogen 96** 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: *cador* pathogen protocol or *cador* pathogen heated 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 29.
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 **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 discard 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 VXL, proteinase K, carrier RNA and internal control (if applicable), according to Table 2 on page 27.

    Important: Prepare VXL mixture immediately before starting the run.
    For the *cador* pathogen protocol place the VXL mixture in the indicated positions on the worktable.
    For the *cador* pathogen heated off-board lysis protocol, you will need the VXL mixture during the manual lysis procedure.

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

	Number of samples									
	24	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**
Buffer VXL (ml)	4.6	5.9	7.4	8.8	9.9	11.8	12.7	14.1	15.5	17
Proteinase K (µl)	580	740	920	1100	1240	1470	1600	1800	2000	2200
Carrier RNA* (µl)	30	40	46	56	62	74	80	90	96	100
Internal Control* (µl)	280	370	460	550	620	730	800	880	980	1000

*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 *cador* pathogen protocol, add 200 µl of each sample per well.

    For the *cador* pathogen heated 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.

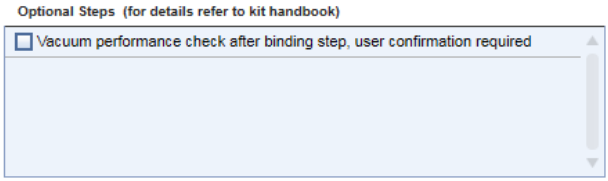
   Note: We recommend not reusing reagents for multiple runs.
6. Remove the input plate.
7. Discard the QIAamp 96 plate or keep partially used QIAamp 96 plates for subsequent reuse. In this case cover used fractions with an adhesive tape.
8. Remove the tip chute and all adapters from the worktable. Remove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

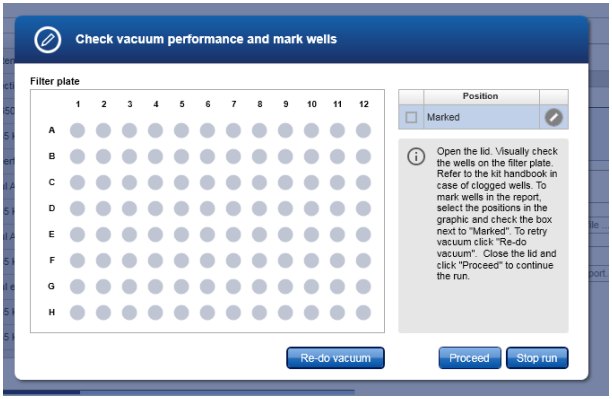
Optional steps

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 to the user to observe the wells.

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

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

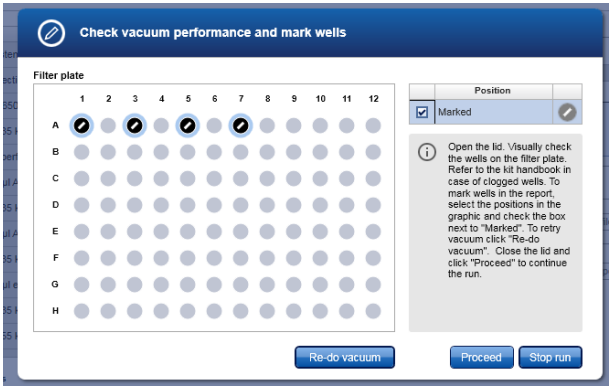
   If liquid remains in the wells, click the **Re-do vacuum** button to apply the same vacuum pressure again. The vacuum will be activated for a certain time or until you press the **Stop vacuum** button.

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

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

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

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



4. If liquid still remains in any well, manually remove the liquid using a pipet.

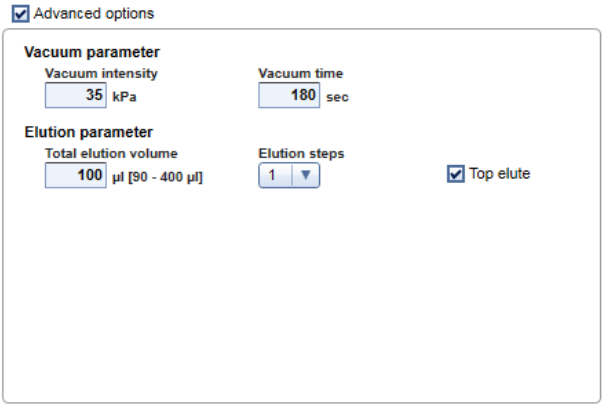
5. After the instrument has added additional reagents, open the hood to pause the run. Check to see whether the affected well is still blocked. If so, manually remove the liquid from the affected well using a pipet.
6. Either click **Proceed** to continue the run, or click **Stop run** to stop the run.

Advanced options

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

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

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



Vacuum parameter

In the **Vacuum parameter** section, it is possible to change two parameters: vacuum intensity and vacuum time. The default settings are 35 kPa for the vacuum intensity and 180 sec for the vacuum time.

The vacuum intensity can be changed from 25 kPa to 70 kPa. Please note that these changes are not recommended by QIAGEN. Changing the vacuum intensity parameter only affects the vacuum intensity following the binding step. All other vacuum steps will be unaffected.

The vacuum time can be changed from 30 sec to 600 sec. Please note that these changes are not recommended by QIAGEN. Changing the vacuum time parameter only affects 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. The total elution volume can be changed 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.

The elution parameter can be increased 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 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 44).
- 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 fluid sample.

   Note: If starting samples are less than 400 µl, 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.

4. Remove the Pathogen Lysis Tube from the vortexer and briefly centrifuge the tube to remove liquid from the inside of the lid.

   Note: If solid particles are expected in the samples, centrifuge at 6000 *x g* for 1 min.

5. Use 200 µl of the supernatant as starting material and proceed directly with "cador Pathogen Protocol", page 22.

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

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

Important points before starting

- Buffer ATL and Pathogen Lysis Tubes L (including Reagent DX) must be ordered separately (for ordering information, see page 44).
- 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 of each fluid sample to a Pathogen Lysis tube and centrifuge for 5 min at maximum speed (>14,000 *x g*).

2. Remove the supernatant using a pipet and discard the supernatant. Repeat steps 1 and 2, if necessary.
3. Add 500 µl Buffer ATL (containing Reagent DX) and resuspend each 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.
5. Remove the Pathogen Lysis Tube from the vortexer and briefly centrifuge the tube to remove liquid from the inside of the lid.
6. Use 200 µl of the supernatant as starting material and proceed directly with "cador Pathogen Protocol", page 22.

Pretreatment O1 for oral fluids

Procedure

1. Add 400 µl of each oral fluid sample to 2 ml microcentrifuge tubes.
2. Centrifuge the samples at 9000 *x g* for 5 min at room temperature (15–25°C).
3. Use 200 µl of the supernatant as starting material and proceed directly with "cador Pathogen Protocol", 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 (see "Ordering Information" on page 44.

Procedure

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

   Note: 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 into 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 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 "cador Pathogen Protocol", page 22.

   Note: 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 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 (see "Ordering Information" on page 44)

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.

   Note: 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 in this procedure, carrier RNA and QIAGEN Proteinase K can be omitted from the VXL mixture (Table 2, page 27). 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 the QIAGEN Proteinase K digest and/or increase the amount of QIAGEN Proteinase K to 40 µl. Reduce the amount of starting material in future preparations of this tissue type.

If no thermal mixer, 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 step 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* for 1 min. Use 200 µl of the supernatant in the next step.

   Important: Ensure that no solid particles are transferred to the next step.

5a. For isolation of viral DNA or DNA from easy-to-lyse bacteria, proceed directly with the "cador Pathogen Protocol", 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 for difficult-to-lyse bacteria in whole blood or pretreated tissue" on page 34.

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 and proceed with the "cador Pathogen Protocol", page 22. If using a lower sample volume, adjust to 200 µl with PBS or water.

   Important: Ensure that no solid particles are transferred to the purification protocol.

Pretreatment F2 for isolation of bacterial and viral DNA from feces

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 44).

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 the 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 and proceed with the "cador Pathogen Protocol", page 22. If using a lower sample volume, adjust to 200 µl with PBS or water.

   Important: Ensure that no solid particles are transferred to the purification protocol.

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	When processing samples that might potentially block the membrane, we recommend using a vacuum performance check. 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 wash buffer, open the hood to pause the run. Check if the well is still blocked. If so, remove all liquid using a pipet. 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).

		Comments and suggestions
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.
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, use the heated off-board lysis protocol.
d)	Carrier RNA not added to Buffer VXL mix 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.
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.

		Comments and suggestions
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.
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 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.

Ordering Information

Product	Contents	Cat. no.
cador Pathogen 96 QIAcube HT Kit	For 480 preps: QIAamp 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*	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*	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
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

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

† Larger Kit sizes available; please inquire.

Product	Contents	Cat. no.
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

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

- cador *Pathogen 96 QIAcube HT Handbook:* **www.qiagen.com/HB-2166**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for the purification of pathogen nucleic acids from various samples. See the cador *Pathogen QIAcube HT Handbook* for sample pretreatments.
- Prepare Buffers ACB, AW1, AW2 and carrier RNA according to the instructions in the cador *Pathogen 96 QIAcube HT Handbook*.
- Prepare a mixture of Buffer VXL, carrier RNA, proteinase K 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 *cador* Pathogen 96 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: *cador* Pathogen protocol (including lysis) or Heated 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 VXL mixture preparation.

Samples	24	32	40	48	56	64	72	80	88	96
Buffer VXL (ml)	4.6	5.9	7.4	8.8	9.9	11.8	12.7	14.1	15.5	17
Proteinase K (µl)	580	740	920	1100	1240	1470	1600	1800	2000	2200
Carrier RNA* (µl)	30	40	46	56	62	74	80	90	96	100
Internal Control* (µl)	280	370	460	550	620	730	800	880	980	1000

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

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