

IndiSpin® QIAcube® HT Pathogen Kit Handbook

For automated purification of viral RNA and DNA and bacterial DNA from animal whole blood, serum, plasma, body fluids, oral fluids, swabs and washes, tissue and feces



IndiSpin QIAcube HT Pathogen Kit (cat no SP54161),
formerly cador® Pathogen 96 QIAcube HT Kit



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Kit contents

IndiSpin QIAcube HT Pathogen Kit	(480)
Cat. no.	SP54161
Number of preps	480
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IndiSpin 96 Plate	5
Buffer VXL ¹	3 x 30 ml
Buffer ACB (concentrate) ^{1,2}	2 x 60 ml
Proteinase K	2 x 6 ml
Carrier RNA (poly A)	2 x 310 µg
Buffer AW1 (concentrate) ^{1,3}	2 x 98 ml
Buffer AW2 (concentrate) ³	2 x 66 ml
Buffer AVE	2 x 125 ml
TopElute Fluid	1 x 60 ml
Quick-Start Protocol (PCard)	2

1 CAUTION: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 5 for safety information.

2 Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.

3 Before using for the first time, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.

Storage

IndiSpin 96 plates and buffers can be stored dry at room temperature (15-25°C) until the expiration date stated on the kit box without affecting performance.

Lyophilized Carrier RNA can be stored at room temperature until the expiration date stated on the kit box. For use, lyophilized Carrier RNA should be dissolved in Buffer AVE and then added to Buffer VXL, as described in “Preparing reagents”, on page 19. This Carrier RNA/Buffer AVE/Buffer VXL mix solution should be prepared fresh and is stable at room temperature for up to 48 hours. Unused Carrier RNA dissolved in Buffer AVE should be immediately frozen in aliquots at -30 to -15°C. Do not subject aliquots of Carrier RNA to more than 3 freeze-thaw cycles.

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 IndiSpin QIAcube HT Pathogen Kit is intended for the extraction of pathogen nucleic acids (viral RNA and DNA, and bacterial DNA) from animal whole blood, serum, plasma, body fluids, oral fluids, swabs, washes, tissue (homogenate), and feces.

For molecular biology applications.

Symbols



Legal manufacturer



Lot number



Use by date



Temperature limitations for storage



Handbook



Catalog number



Material number

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 from your local sales representative or by Email request under compliance@indical.com.



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 INDICAL's ISO-certified Quality Management System, each lot of IndiSpin QIAcube HT Pathogen Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The IndiSpin QIAcube HT Pathogen Kit enables the efficient purification of viral RNA and DNA, as well as bacterial DNA, from a broad range of animal sample types, including whole blood, serum, plasma, swabs, washes, tissue, and feces (see “Starting material” on page 14). 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.

However, specific combinations of sample types and pathogens should be validated by the user.

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 (15-25°C) in the presence of Proteinase K and Buffer VXL, which together ensure the inactivation of nucleases. Adding Buffer ACB adjusts the binding conditions for the copurification of DNA and RNA. The lysate is then transferred to an IndiSpin 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 pathogen species and must be validated by the user. Some samples may require a pretreatment (see Table 1, page 10).

Nucleic acid purification protocol

This handbook contains two protocols describing the purification of pathogen nucleic acids from different sample types:

- For use with QIAcube HT 4.17 software (starting page 27).
- For use with QIAcube HT Prep Manager (starting page 31).

The purification of pathogen nucleic acids 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
- oral fluid
- 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*

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. For samples that require a pretreatment prior to nucleic acid purification, Table 1 on page 11 provides an overview of which pretreatment protocols are suited to different starting material and pathogen combinations.

* The processing of samples with a high inhibitor content, such as urine and feces, may require a reduction in sample input volume or further measurements. For further pretreatment recommendations, contact INDICAL support (support@indical.com).

The lysis and binding solutions used in the procedure are Buffer VXL and Buffer ACB. Please pay attention to the information given under “Safety information”, page 6.

Pretreatments

The pretreatments mentioned in this handbook are optimized for specific combinations of starting material and target pathogens. The choice of pretreatment depends on the workflow focus and is to be followed by nucleic acid purification.

Table 1 on page 11 summarizes the pretreatments and their applications.

Some of the pretreatments may require additional components, which are indicated in each pretreatment protocol.

Table 1: Overview of available pretreatment protocols

Sample	Target	Pretreatment	handbook
Fluids (e.g., whole blood, serum, plasma, swab or wash fluid, pretreated tissue)	Viral RNA and DNA, DNA of easy-to-lyse bacteria ¹	-	-
Whole blood or pretreated tissue	DNA of difficult-to-lyse bacteria ¹	Pretreatment B1 for difficult-to-lyse bacteria in whole blood or pretreated tissue	HB-2533
Serum, plasma, swabs, washes, body cavity fluids, urine	DNA of difficult-to-lyse bacteria ¹	Pretreatment B2 for difficult-to-lyse bacteria in body fluids ²	HB-2534
High volume cell-free fluids (for increased sensitivity)	DNA of easy-to-lyse bacteria ¹	Pretreatment B3 for difficult-to-lyse bacteria in body fluids ²	HB-2549

Tissue (e.g., liver, spleen, kidney, lymph node)	Pathogen nucleic acids	Pretreatment T1 Mechanical disruption of tissue	HB-2535
	Viral DNA ³ , bacterial DNA ⁴	Pretreatment T2 Enzymatic digestion of tissue	HB-2536
Rapid Partial Disruption of tissue	Viral RNA and DNA, DNA of easy-to- lyse bacteria ¹	Pretreatment T3	HB-2537
Tissue containing high amount of lipids and/or nucleases (e.g. brain, pancreas)	Viral RNA and DNA, DNA of easy-to- lyse bacteria ¹	Pretreatment T4	HB-2538
Feces	Viral RNA and DNA	Pretreatment F1 Non-lysing suspension method	HB-2513
	Bacterial DNA ¹ and viral DNA	Pretreatment F2 Lysing suspension method	HB-2514
	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP) DNA	Pretreatment F-MAP	HB-2503
Filter paper cards		Pretreatment C1	HB-2520
Swabs (tracheal, oropharyngeal, blood)		Pretreatment S1	HB-2516
Oral fluids		Pretreatment O1	HB-2551

1 Gram-positive bacteria are difficult to lyse due to their rigid cell wall. Many Gram-negative bacteria are easy to lyse, but some are not and will also benefit from Pretreatment B1 or B2.

2 Not suitable for whole blood.

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

4 For difficult-to-lyse bacteria, subsequently use Pretreatment B1.

For further information on Pretreatments website
www.indical.com/handbooks contact INDICAL Support at
support@indical.com.

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.

- Pipettors and disposable pipette tips with aerosol barriers (20-1000 µl)
- Ethanol (96-100%)*
- Isopropanol
- Phosphate-buffered saline (PBS), may be required for diluting samples
- Vortexer
- QIAcube HT instrument
- QIAcube HT Plasticware
- QIAcube HT 4.17 Software or QIAcube HT Prep Manager Software
- QIAcube HT Reagent troughs

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

Important notes

Starting material

Do not overload the IndiSpin 96 Plate, 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). When working with difficult samples, use a vacuum performance check to check if all liquid has passed the membrane.

Highly viscous fluids may require treatment to reduce their viscosity, to allow for efficient extraction of pathogen nucleic acids. Please contact INDICAL support at **support@indical.com** 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 freeze-thawed more than once. Freeze-thawing more than once can lead to denaturation and

precipitation of proteins, resulting in potential reduction in viral titers, and therefore, reduced yields of viral nucleic acids.

After collection and centrifugation, 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 at -70°C.

We recommend using 50-200 µl blood containing non-nucleated erythrocytes. 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 less than 50 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 IndiSpin 96 Plate 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 must be used in the nucleic acid purification protocol to prevent the loss of nucleic acids during the procedure (see page 17 for information on 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, e.g., 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 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. Therefore, suitability of the pretreatment protocols in this handbook should be evaluated for each new combination of tissue and pathogen. Additional pretreatments for tissue samples are available at INDICAL Support, 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 and plasma), the yield of viral 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 IndiSpin QIAcube HT Pathogen Kit 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 to the silica membranes, which is especially important when the target molecules are not abundant. In addition, an excess of Carrier RNA reduces the chances of viral RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergents in the lysis buffer. Not

using Carrier RNA may decrease the recovery of viral nucleic acids. Do not add Carrier RNA to whole blood and tissue samples, or other samples containing a high amount of cells.

Internal Control

Use of an internal control, such as the in type IC-DNA or in type IC-RNA is optional, depending on the amplification system of choice. If the IndiSpin QIAcube HT Pathogen Kit is used in combination with amplification systems that employ an internal control, introduction of these internal controls may be required 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 or contact INDICAL Support (support@indical.com) for further information.

Storing nucleic acids

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

Handling RNA

RNases are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Preparing reagents

Carrier RNA stock solution

For use, lyophilized Carrier RNA should first be dissolved in Buffer AVE. Add 310 μ l Buffer AVE to the tube containing 310 μ g lyophilized Carrier RNA to obtain a stock solution of 1 μ g/ μ l. Add this solution to Buffer VXL mixture as in Table 2 on page 28 or Table 3 page 32. 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.

Adding Carrier RNA to Buffer VXL

We recommend adding Carrier RNA to fluids containing a low amount of cells such as serum, plasma, swabs media, and wash fluid. Do not add Carrier RNA to samples with a high cell content such as whole blood and tissue because high amounts of background nucleic acids may negatively influence downstream applications such as RT-PCR.

Carrier RNA dissolved in Buffer AVE is added to Buffer VXL so that 1 μ g carrier RNA is present in each sample.

Note: 100 µl of Buffer VXL containing dissolved Carrier RNA is used per preparation.

Important note: Carrier RNA does not dissolve in Buffer VXL. It must first be dissolved in Buffer AVE.

The Buffer VXL solution containing dissolved Carrier RNA should be prepared fresh and is stable at room temperature (15-25°C) for up to 48 hours.

Proteinase K

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

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 Proteinase K at 2-8°C.

Add 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. 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. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature (15-25°C) for up to 1 year. 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. 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. When handling Buffer AVE, take extreme care to 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 IndiSpin 96 Plate 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 IndiSpin 96 Plate.

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 on page 24 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 IndiSpin 96 plate in the transfer carriage. Load the carriage with the IndiSpin 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.

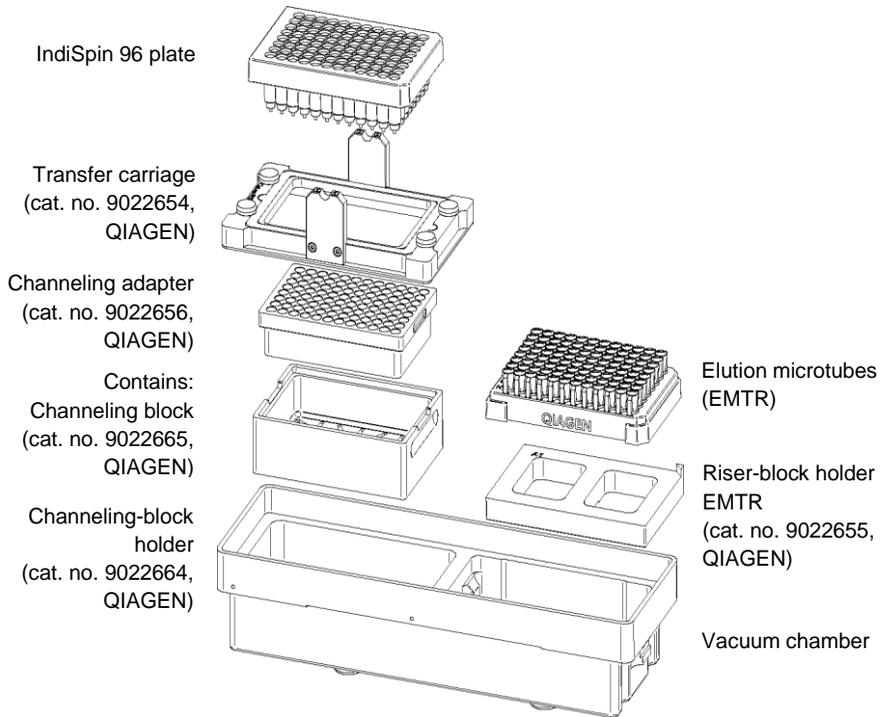


Figure 1: Assembling the vacuum chamber

Optional features

Processing fewer than 96 samples per run

If processing fewer than 96 samples reuse of IndiSpin 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 IndiSpin 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.

If lysis with Proteinase K is carried out off-board, Proteinase K may be exchanged with water when setting up the worktable.

QIAcube HT 4.17 Software

A protocol allowing for lysis outside the instrument is available from our technical support team.

QIAcube HT Prep Manager Software

When using an off-board lysis protocol, choose the cadour pathogen

heated off-board lysis protocol in the software setup step under the selected protocol.

Manual lysis procedure

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

1. Add 200 μ l of each fluid sample to the bottom of an S-Block well.
2. Cover the S-Block with adhesive tape.
3. Incubate at 70°C, with constant agitation, for 10–15 min.
4. Optional: Briefly centrifuge the S-block to remove liquid from the inside of the tape.
5. Remove the adhesive tape from the S-Block.
6. Proceed with the “*cador* pathogen protocol on the QIAcube HT”, using either the QIAcube HT 4.17 software (page 27) or the QIAcube HT Prep Manager software (page 31).

Protocol for use with QIAcube HT 4.17 software

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

- Before beginning the procedure, read “Important notes” (page 14).
- Do not overload the IndiSpin membranes as this can lead to impaired nucleic acid extraction and / or performance in downstream applications.
- Avoid repeated freezing and thawing of samples as this may reduce nucleic acid yield and quality.

Things to do before starting

- If necessary, thaw and equilibrate samples at 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” (page 19).
- When working with difficult samples, use a vacuum performance check to check if all liquid has passed the membrane. See

Troubleshooting Guido on page 40 and the QIAcube HT User Manual for guidance.

- Ensure that the correct software is installed.
- Ensure that the relevant version of the of the *cador Pathogen 96 QIAcube HT mit EtOH 180µl VXL.QSP* run file is installed on the instrument.
- Ensure that you are familiar with operating the instrument. Refer to the QIAcube HT User Manual for operating instructions.
- If the volume of the sample is less than 200 µl, add PBS or 0.9% NaCl to a final volume of 200 µl.
- If necessary, prepare a mixture of Buffer VXL and Carrier RNA according to Table 2 on page 28, for use in step 8 of the procedure.

Note: Prepare a volume of the Buffer VXL/Carrier RNA/Internal Control mixture that is 10% greater than that required for the total number of sample purifications to be performed.

Table 2: 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 Internal Control or Carrier RNA, use RNase-free water instead.

Procedure

1. Place the tip discard chute on the worktable so that the chute is over the tip disposal box.
2. Switch on the instrument. The switch is located at the lower left of the backside of the instrument. Launch the QIAcube HT Software and select the “Recent” tab.
3. To open the run file, select the protocol “*cador Pathogen 96 QIAcube HT mit EtOH 180µl VXL*” and then click “Open”. For more information about optional steps and advanced options see the kit handbook.
4. Click  on the toolbar.
5. Select the appropriate number of samples arranged in columns in the 96-well plate. Ensure that the “*Turn the HEPA filter on automatically*” option is checked and click “Jump to End”. Confirm the protocol by clicking “Finish”. The wizard closes.
6. Prepare the vacuum chamber (chapter “Assembling the vacuum chamber”, page 23).

Note: If fewer than 12 columns are to be processed, seal unused columns of the IndiSpin 96 plate with adhesive tape (supplied).
7. Add 200 µl sample to the selected S-Block wells. Place the S-Block in the B1 position of the instrument’s worktable.
8. 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. Prepare the indicated volume of the Buffer VXL mixture and mix well. Start the run immediately and perform the pre-run check.
9. After completing the pre run check, close the instrument hood and click “OK”.
10. Cover the elution plate (EMTR) with the lid and remove from the elution chamber, when the protocol is complete.

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.

11. Create a report (if required).
12. Discard used plasticware and the Buffer VXL mixture. We recommend discarding leftover reagents in the reagent troughs.
13. Clean the instrument following instructions below.

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 IndiSpin 96 plate or keep partially used IndiSpin 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 QIAcube HT User Manual.

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

Protocol for use with QIAcube HT Prep Manager software

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

- Before beginning the procedure, read “Important notes” (page 14).
- Do not overload the IndiSpin membranes as this can lead to impaired nucleic acid extraction and / or performance in downstream applications.
- Avoid repeated freezing and thawing of samples as this may reduce nucleic acid yield and quality.

Things to do before starting

- If necessary, thaw and equilibrate samples at 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” (page 19).
- When working with difficult samples, use a vacuum performance check to check if all liquid has passed the membrane. See

Troubleshooting Guide on page 40 and the QIAcube HT User Manual for guidance.

- Ensure that the correct software is installed.
- Ensure that the relevant version of the of the *cador Pathogen 96* run file is installed on the instrument.
- Ensure that you are familiar with operating the instrument. Refer to the QIAcube HT User Manual for operating instructions.
- If the volume of the sample is less than 200 μl , add PBS or 0.9% NaCl to a final volume of 200 μl .
- If necessary, prepare a mixture of Buffer VXL and Carrier RNA according to Table 3 on page 32, for use in step 10 of the procedure.

Note: Prepare a volume of the Buffer VXL/Carrier RNA/Internal Control mixture that is 10% greater than that required for the total number of sample purifications to be performed.

Table 3: 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 Internal Control or Carrier RNA, use RNase-free water instead.

Procedure

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).
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 defining 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.

Cleaning the instrument after completing a run

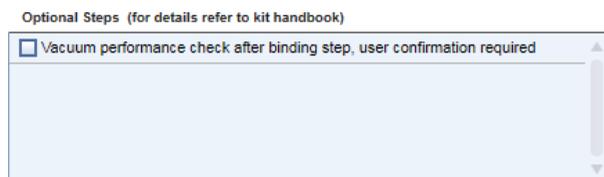
1. Follow the instructions in the QIAcube HT Prep Manager Software for cleaning the instrument after a run.
2. Cover tip racks that contain only unused tips with the lid and remove them from the worktable.
3. Cover fractions of partly used tip racks with an adhesive tape. Then cover the tip racks with 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 IndiSpin 96 plate or keep partially used IndiSpin 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.

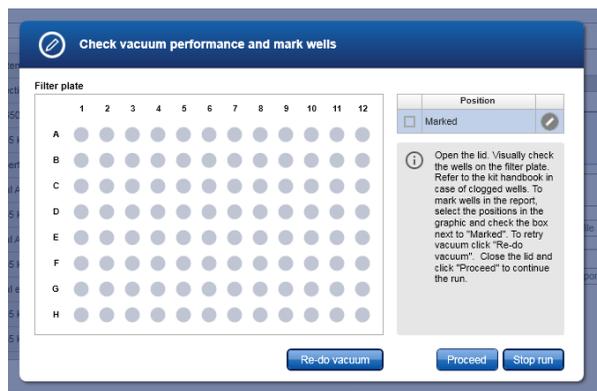
Optional steps

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 IndiSpin 96 plate for any remaining liquid.

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

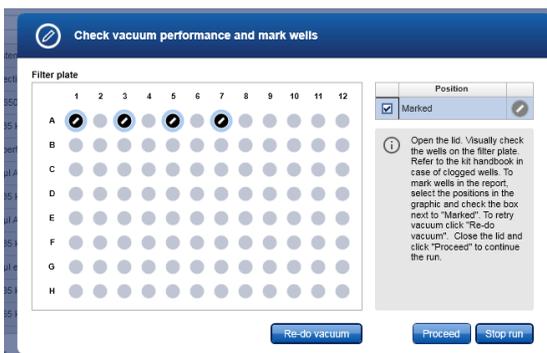
If liquid remains in the wells, click the **Re-do vacuum** button to apply the same vacuum pressure again. The vacuum will be activated for a certain time or until you press the **Stop vacuum** button.

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 IndiSpin 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: INDICAL and QIAGEN do 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. INDICAL and QIAGEN are not responsible for the outcome and do not support experiments performed using modified advanced options. Please note that any changes to these options are carried out at your own risk.

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

Advanced options

Vacuum parameter

Vacuum intensity <input type="text" value="35"/> kPa	Vacuum time <input type="text" value="180"/> sec
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Elution parameter

Total elution volume <input type="text" value="100"/> μl [90 - 400 μl]	Elution steps <input type="text" value="1"/> ∇
--	--

Top elute

Vacuum parameter

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

The vacuum intensity can be changed from 25 kPa to 70 kPa. Please note that these changes are not recommended by INDICAL and 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 INDICAL and 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 INDICAL and 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 INDICAL and QIAGEN.

TopElute

TopElute Fluid is used during elution of nucleic acids from the IndiSpin 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 IndiSpin 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 INDICAL and 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.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise.

For more information or help please contact INDICAL Support at support@indical.com.

Comments and suggestions	
Instrument issues	
1 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.
2 Instrument failure/ cancelled run	It is not possible to recover the run. Please continue manually
3 Blocked membranes	<p>When processing samples that might potentially block the membrane, we recommend using a vacuum performance check.</p> <p>If liquid is still visible, remove 500 µl using a pipet. Then scrape the surface of the membrane with a fresh pipet tip in order to relocate any solid particles that may block the membrane. Take care not to damage the membrane. If there is still no liquid flow, pipet all liquid from the well and proceed with the run.</p> <p>After the instrument has added wash buffer, open the hood to pause the run. Check if the well is still blocked. If so, remove all liquid using a pipet. Do not perforate the membrane. Uncovered perforated wells will disturb vacuum integrity during elution across the whole plate.</p> <p>Proceed the run. No buffer will float over from the blocked well into other wells from this step on.</p> <p>Next time, use less sample (tissue).</p>

Little or no pathogen DNA or RNA in the eluate		
1	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.
2	Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW or Buffer AW2 concentrate was diluted with the correct volume of 96–100% ethanol, as indicated on the bottle. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification protocol with new samples.
3	Insufficient sample lysis	<p>Proteinase K was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh Proteinase K (see storage recommendations on page 5).</p> <p>For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. In this case, mix the samples thoroughly after addition of Proteinase K and incubate for 15 min at 70°C.</p>
4	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.
5	Buffer VXL/Carrier RNA mixture mixed insufficiently	Mix well by pipetting with a large pipette.
6	RNase contamination in Buffer AVE	If tubes containing Buffer AVE are accessed repeatedly, be careful to not introduce RNases which can degrade viral RNA. In case of RNase contamination, replace the open vial of Buffer AVE with a new vial. Repeat the purification procedure with new samples.
7	Nucleic acids in samples already degraded prior to purification	Samples were freeze-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	
1	<p>Little or no DNA or RNA in the eluate</p> <p>See “Little or no pathogen DNA or RNA in the eluate” (above) for possible reasons.</p>
2	<p>Excessive eluate in the amplification reaction</p> <p>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 might not be complete without special treatment. Reduce the amount of sample input or/and the amount of eluate added to the amplification reaction.</p>
3	<p>Too much background nucleic acids in the eluate</p> <p>Determine the maximum amount of Carrier RNA suitable for your amplification reaction. Adjust the concentration of Carrier RNA solution added to the Buffer VXL accordingly. In RT-PCR, a low DNA background is preferable. Use less eluate</p>
4	<p>Performance of purified nucleic acids in assays varies with aging of reconstituted wash buffers</p> <p>Salt and ethanol components of Buffer AW1 or Buffer AW2 may have separated out after being left for a long period between preparation. Always mix buffers thoroughly before each preparation.</p>
Precipitate in buffers	
1	<p>Precipitate in Buffer VXL or Buffer ACB</p> <p>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.</p>

Order information

Product name	Cat. no.
IndiSpin QIAcube HT Kit (480) <i>formerly cadorr Pathogen QIAcube HT Kit</i>	SP54161
QIAcube HT PW (480) <i>formerly QIAcube HT Plasticware</i>	PW950067
intype IC-DNA	IC289980
intype IC-RNA	IC289970

INDICAL offers a broad range of ready-to-use pathogen specific ELISA kits, qPCR/ RT-qPCR assays and reagents.

To optimize your workflow, and to handle your sample and throughput needs, INDICAL additionally offers instruments and kits for the efficient extraction of nucleic acids from a variety of sample types.

Visit www.indical.com for more information about bactotype, cadorr, cattletype, flocktype, IndiMag, IndiSpin, intype, pigtype and virotype products.

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Change index

Handbook	Version	Change
HB-2166-EN-002	January 2021	INDICAL design