Supplementary Protocol

QIAamp® 96 Viral RNA Kit

This protocol is for use with QIAcube® HT Prep Manager Software (version 1.1 or higher). The QIAamp 96 Viral RNA Kit is for the extraction of total RNA from human respiratory swab samples. The QIAamp 96 Viral RNA Kit (cat. no. 52962) can be stored at room temperature (15–25°C) for up to 12 months.

The kit configuration contains two protocols:

- QIAamp Viral RNA protocol
- QIAamp Viral RNA off-board lysis protocol

  Note: The QIAamp Viral RNA off-board lysis protocol starts with the addition of 560 µL ethanol.

Further information

- *QIAamp Viral RNA Handbook* for the manual procedure: **www.qiagen.com/HB-2814**
  The handbook describes the manual procedure
- QIAcube HT protocol file: **https://www.qiagen.com/us/products/diagnostics-and-clinical-research/sample-processing/qiaamp-viral-rna-kits/**
- *QIAcube HT User Manual*: **www.qiagen.com/HB-2120**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Equipment and reagents to be supplied by the 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.

- Ethanol (96–100 %)
- QIAcube HT Instrument with Prep Manager Software
- QIAcube HT protocol file (QCHT_KitConfig_QIAamp96ViralRNA_v1-0-21.xml)
- QIAcube HT reagent troughs
  Reagent Trough (with lid), 70 mL (cat. no. 990554)
  Reagent Trough (with lid) 170 mL (cat. no. 990556)

- QIAcube HT Plasticware (cat. no. 950067)
- Optional:
  - Elution Microtubes RS (24 x 96) (cat. no.120008)
  - S-Blocks (24) (cat. no.19585)
  - Filter-Tips, OnCor C, 200 µL (10 x 96) (cat. no. 990610)

Note: The alternative plastic material mentioned in the *QIAamp 96 Viral RNA Handbook* is not suitable for use on the QIAcube HT.

Notes before starting

This protocol is for the purification of viral nucleic acids from nasotracheal swab samples using the QIAamp Viral RNA Kit with the QIAcube HT Prep Manager Software.

- Equilibrate buffers to room temperature (15–25°C).
- Prepare buffers AW1 and AW2 according to the instructions in the *QIAamp 96 Viral RNA Handbook*.

Preparation of carrier RNA-Buffer AVL solution

1. Add 1550 µL Buffer AVE to the tube containing 1550 µg lyophilized carrier RNA to obtain a solution of 1 µg/µL.
2. Check Buffer AVL for precipitate, and if necessary, incubate at 80°C until the precipitate is dissolved.
3. Prepare a mixture of Buffer AVL, carrier RNA, and internal control (if applicable) according to Table 1 below.

Table 1. Volumes of Buffer AVL and Buffer AVE–carrier RNA mix required for the QIAamp 96 Viral RNA procedure

No. of samples	Buffer AVL (mL)	Buffer AVE–carrier RNA (mL)
24	15	150
32	20	200
40	24	240
48	29	290
56	34	340
64	39	390
72	44	440
80	48	480
88	53	530
96	58	580

Note: Buffer AVL values and Buffer AVE–carrier RNA values are rounded up to simplify pipetting.

4. Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

Preparation of samples

Swabs should be placed immediately into a sterile transport tube containing 2–3 mL of either viral transport medium (VTM) or sterile saline. If particles are visible, the swabs should first be liquefied or centrifuged for 10 min at 1500 x *g* to avoid clogging during isolation procedure. Transfer 140 µL sample into the S-block.

QIAamp 96 Viral RNA 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 time.

2. Start the QIAcube HT Prep Manager software. Click on the Home icon in the main toolbar to access the home screen.

3. Select "QIAamp 96 Viral RNA" from the **Create experiment** list. Follow the instructions in the wizard and fill in all required fields.

4. In the **Setup** step, select "Fluid sample" as **Sample type** and "any" unde**r Pre-treatment**.

5. Select the protocol: QIAamp Viral RNA protocol.
   Note: "Vacuum performance check" is checked by default
   Further information on Vacuum performance check and Advanced options are at the end of this document.

6. Click **Next Define** samples in the **Labware selection** step.

7. 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.

8. Follow the instructions on the virtual worktable to prepare the instrument worktable. Sample volume is 140 μL

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** step has been selected, the software will show a dialog that needs to be confirmed after the 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 TopElute fluid, there may be two liquid phases in the elution microtubes. TopElute fluid will be the top layer, above the elution buffer.

12. Create a report (if required).

13. Follow the cleaning procedure.

QIAamp viral RNA off-board lysis protocol on the QIAcube HT

The lysis of the samples have to be performed manually (off-board). The protocol starts with addition of ethanol to the lysed samples.

Sample preparation

1. Pipette 560 μL Buffer AVL containing carrier RNA into each well of the S-Block.

2. Add 140 μL sample to each well by touching the insides of the wells without wetting the rims of the wells. Mix thoroughly by pipetting up and down 8 times.
   Note: To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer AVL to yield a homogeneous solution.

3. Seal the S-Block with a tape pad and incubate at room temperature (15–25°C) for 10 min.

Preparing QIAcube HT

1. Switch on the QIAcube HT instrument and the connected laptop computer. We recommend leaving the vacuum station switched on at all time.

2. Start the QIAcube HT Prep Manager software. Click on the Home icon in the main toolbar to access the home screen.

3. Select "QIAamp 96 Viral RNA" from the **Create experiment** list. Follow the instructions in the wizard and fill in all required fields.

4. In the **Setup** step, select "Fluid sample" as **Sample type** and "any" under **Pre-treatment**.

5. Select the protocol: **QIAamp Viral RNA off-board lysis protocol**.

   Note: "**Vacuum performance check**" is checked by default

6. Further information about Vacuum performance check and advanced options are at the end of this documents.

7. Click **Next Define** samples in the **Labware selection** step.

8. 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.

9. Follow the instructions on the virtual worktable to prepare the instrument worktable. Sample volume is 700 µL (in the S-Block from step 3 in the "Sample preparation" section).

10. Save the experiment by clicking the **Save** button in the button bar.

11. Click the **Start run** button to start the run.

    Important: If the optional **Vacuum performance** step has been selected, the software will show a dialog that needs to be confirmed after the defined vacuum steps.

12. When the protocol is complete, cover the elution plate (EMTR) with the lid and remove it from the elution chamber.

    Note: If using TopElute fluid, there may be two liquid phases in the elution microtubes. TopElute fluid will be the top layer, above the elution buffer.

13. Create a report (if required).

14. 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 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 checked.
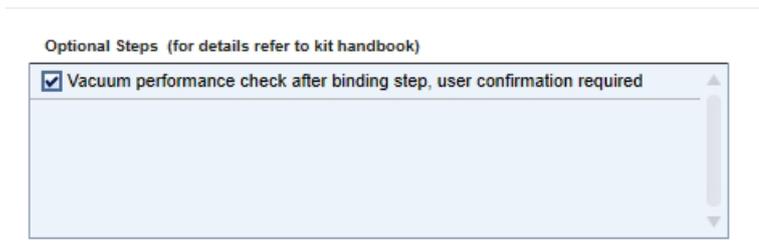
Figure 1. Vacuum performance check is enabled.

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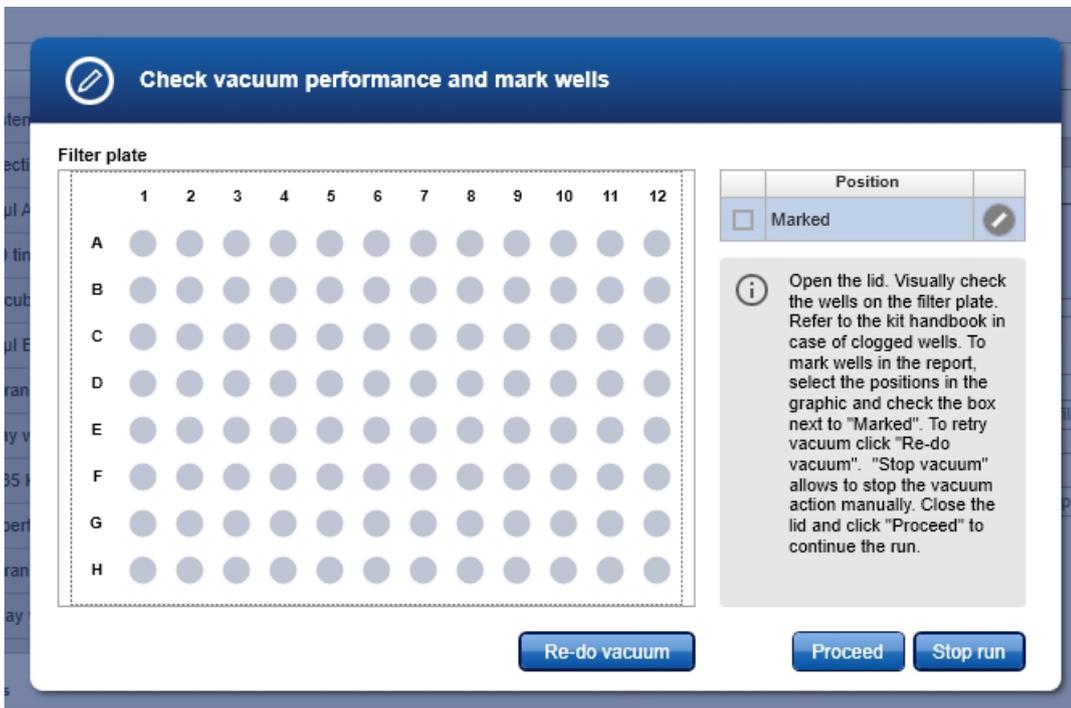

Figure 2. Prompt appears to let the user choose to re-do vacuum or continue the run.

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**.
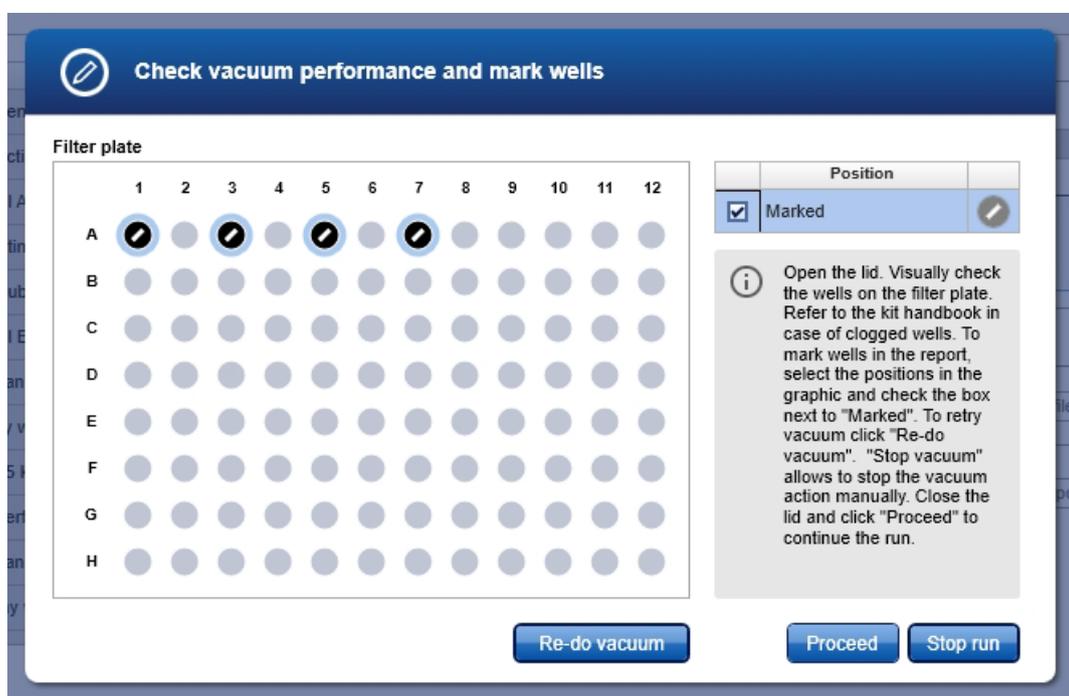

Figure 3. User can mark or unmark well positions.

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

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 pipette.

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 by QIAGEN 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.

Figure 4. Vacuum and elution parameters can be modified. QIAGEN does not recommend changing any of the parameters in the Advanced options.

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

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

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

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

Elution parameter

In the **Elution parameter** section, it is possible to change the total elution volume and the elution step. The recommended values for these parameters are shown in the QIAcube HT Prep Manager Software. If you want to adapt your protocol you could change the total elution volume to another value within the defined range.

Please note that these changes are not recommended by QIAGEN.

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 TopElute 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.

Document Revision History

Date	Changes
11/2022	Initial revision

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.