Supplementary Protocol November 2020

QIAprep&amp Viral RNA UM Kit

This supplementary protocol is for the detection of SARS-CoV-2 using the QIAprep&amp Viral RNA UM Kit (cat. nos. 221415 and 221417) in combination with the SARS-CoV-2 N1+N2 Assay Kit (cat. nos. 222015 and 222017) in epidemiological research. This supplementary protocol is intended for molecular biology application, not for molecular diagnostic use.

Further information

- *QIAprep&amp Viral RNA UM Kit Handbook*: **www.qiagen.com/HB-2830**
- *SARS-CoV-2 N1+N2 Assay Kit Quick-Start Protocol*: **www.qiagen.com/HB-2829**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.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.

- A programmable real-time PCR thermocycler with at least three detection channels, compatible with the QIAprep&amp Viral RNA UM Kit (Table 1)
- Plastic PCR consumables compatible with the abovementioned thermocyclers
- Calibrated micropipettes for volumes ranging from 2 µl to 50 µl and tips or an automated liquid handler

Notes before starting

- The QIAprep&amp Viral RNA UM Kit is an innovative liquid-based method optimized for the preparation and detection of viral RNA targets from samples such as nasal, nasopharyngeal, or oropharyngeal swabs that are stored in non-fixation transport media such as UTM, VTM, PBS, ESwabs®, Viroculт™, or 0.9% NaCl.
- Samples can be kept at room temperature during preparation steps and reaction setup. Sample preparation can conveniently be performed directly in the PCR vessel prior to the addition of the PCR reaction. The assay setup can be done at room temperature and should be processed immediately after sample addition. If heat treatment was performed, storage up to 1 hour at room temperature or for a longer period, frozen at –30 to –15°C, is possible.

- The Viral RNA UM Prep Buffer prepares the samples for the detection step but is not a virus inactivation solution.
- The protocol in this supplementary protocol includes a recommended heat treatment step before the sample preparation step. This workflow step is intended to inactivate the viral particles in an aliquot of the primary sample in transport media (1, 2). QIAGEN cannot guarantee that this heat treatment step will inactivate 100% of viral particles. The inactivation of virus needs to be verified and validated by users. This heat treatment can be substituted by other heat treatments.
- The RT-qPCR protocol uses TaqMan® probes in a multiplex reaction that works with any real-time cyclers. For fluorescence normalization, ROX dye might be required at the following concentrations:
  - **Low concentration of ROX dye**: Applied Biosystems® 7500, ViiA7, and QuantStudio® Real-Time PCR Systems.
  - **High concentration of ROX dye**: ABI PRISM® 7000, Applied Biosystems 7300, 7900, and StepOne® Real-Time PCR Systems.
  - **No requirement for ROX dye**: Rotor-Gene, QIAquant®, Bio-Rad® CFX, Roche® LightCycler® 480, and Agilent® Technologies Mx instruments. The ROX Reference Dye should be used as a 20x concentrated solution for a 1x reaction when using an instrument requiring a high-ROX dye concentration. For instruments requiring a low-ROX dye concentration, use the dye as a 200x concentrate.
- The PCR section of the RT-qPCR protocol must start with an initial incubation step of 2 minutes at 95°C to activate the DNA Polymerase.
- The RNA IC Template + Assay is an inhibition control using a synthetic RNA template. It is a 200 bp IC template detected in the red channel on the Rotor-Gene Q or in the Cy5® channel on other real-time PCR instruments.
- The Human Sampling IC Assay (Sampling Control) is intended to report that the primary sample tube contains intact human genetic material. For this purpose, two different human targets are both detected in the yellow channel on the Rotor-Gene Q or in the VIC®/HEX™ dye channel on other real-time PCR instruments.
- The SARS-CoV-2 N1+N2 Assay Kit contains tubes with a mixture of four primers and two probes purified by HPLC, at a 20x concentration. The four primers are based on the CDC design (**https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html**), targeting N1 and N2 regions of the viral genome. The two probes are coupled with FAM™ as a reporter dye and use ZEN™ quencher for enhanced sensitivity.
- Before use, thaw the Viral RNA UM Prep Buffer, Viral RNA Master Mix, RNA IC Template + Assay, Human Sampling IC Assay, ROX Reference Dye (if required), and RNase-Free Water. Mix the individual solutions.

Table 1. Overview table of the multiplex RT-PCR assay

Assay	Targets	Dye/color channel	Supply
SARS-CoV-2 N1+N2 Assay	N1 and N2 genes	FAM/Green (the two targets detected in the same channel)	SARS-CoV-2 N1+N2 Assay Kit
Inhibition control	Synthetic IVT	Red/Cy5 or similar	QIAprep&amp Viral RNA Kit, optional use but recommended
Sampling control	Human B2M and RNase P genes	HEX/Yellow (the two targets detected in the same channel)	QIAprep&amp Viral RNA Kit, optional use but recommended
Passive reference dye	To be used only for real-time cyclers that require this reference dye	ROX/Orange	QIAprep&amp Viral RNA Kit, optional use

Procedure

1. Prepare a reaction mix according to Table 2 and mix thoroughly.

Table 2. Reaction mix setup

Component	96/384-well block	Final concentration
Viral RNA UM Master Mix, 4x	5 µl	1x
SARS-CoV-2 Assay, 20x	1 µl	1x
RNA IC Template + Assay, 10x	2 µl	1x
Human Sampling IC Assay, 20x	1 µl	1x
ROX Reference Dye (ABI instruments only)	1 µl/0.1 µl*	1x
RNase-Free Water	Fill up to 10 µl	–
Prepared sample (added at step 6)	10 µl	–
Total reaction volume	**20 µl**	**–**

* To be used as a 20x concentrate for high ROX cyclers (i.e., ABI PRISM 7000, Applied Biosystems 7300 and 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for low ROX dye cyclers (i.e., Applied Biosystems 7500, ViiA7, and QuantStudio Real-Time PCR Systems).

2. Vortex the swab containing sample vigorously.
3. Dispense 2 µl of Viral RNA UM Prep Buffer into each PCR tube or well of a PCR plate.
4. **Optional sample heat treatment (recommended)**:
   - 4a. Either the entire primary sample or an aliquot of 50 µl can be submitted for heat treatment. Ensure the complete sample volume is appropriately heated.
   - 4b. Incubate at 70°C for 10 min.
   - 4c. Centrifuge the plate/tube briefly.
5. Transfer 8 µl of the sample to the individual PCR tube or wells containing the Viral RNA UM Prep Buffer. Mix by pipetting up and down at least two times.

6. Incubate at room temperature for 2 min.

   Note: Incubation time starts after adding the last sample to the Viral RNA UM Prep Buffer.

   Do not increase incubation time for more than 6 h.

7. Add 10 µl of the reaction mix prepared in step 1 to the same PCR tubes or wells.

8. **Important consideration**:

   8a. Seal the plate/tube thoroughly to prevent cross-contamination. In case an adhesive film is used, make sure to apply pressure uniformly across the entire plate, to obtain a tight seal across individual wells.

   8b. Mix gently by vortexing for 10–30 s at medium speed. Place the plate in different positions while vortexing, to ensure an equal contact with the vortex platform.

   8c. Centrifuge the plate/tube briefly to collect liquid at the bottom of the plate/tube.

   8d. Immediately proceed to step 9. The complete reaction can be stored only after heat treatment up to 1 h at room temperature or for a longer period, frozen at –30 to –15°C.

9. Place the tubes or plates in the real-time cycler and perform cycling according to the below conditions (Table 3).

   Program the real-time cycler before reaction setup according to Table 3.

   Note: Data acquisition should be performed during the annealing/extension step.

Table 3. Cycling conditions

Step	Time	Temperature	Ramp rate
RT-step	10 min	50°C	Maximal/fast mode
PCR initial heat activation	2 min	95°C	Maximal/fast mode
2-step cycling (40 cycles)			
Denaturation	5 s	95°C	Maximal/fast mode
Combined annealing/extension	30 s	58°C	Maximal/fast mode

10. For results interpretation, refer to Table 4.

Table 4. Possible outcome

Viral RNA assay	Internal control	Sampling control	Status	Result
+	+	+	VALID	Positive
+	+	–	VALID	Positive
+	–	–	VALID	Positive
+	–	+	VALID	Positive
–	+	+	VALID	Negative, virus not detected
–	+	–	Inconclusive	Repeat test using a new sample
–	–	+	PCR inhibited	Repeat test using a lower-sample volume (down to 2 µl)
–	–	–	PCR inhibited	Repeat test using a lower-sample volume (down to 2 µl)

References

1. Batéjat, C., Grassin, Q., Manuguerra, J.-C., and Leclercq, I. (2020). Heat inactivation of the Severe Acute Respiratory Syndrome Coronavirus 2. bioRxiv. https://doi.org/10.1101/2020.05.01.067769.
2. Chin, A.W.H., et al. (2020). Stability of SARS-CoV-2 in different environmental conditions. Lancet. **1**, E10. https://doi.org/10.1016/S2666-5247(20)30003-3

Ordering Information

Product	Contents	Cat. no.
QIAprep&amp Viral RNA UM Kit (600)	For 600 x 20 µl reactions: 1.2 ml Viral RNA UM Prep Buffer; 2 x 1.5 ml Viral RNA Master Mix, 4x; 1.2 ml RNA IC Template + Assay; 0.6 ml Human Sampling IC Assay; 1 ml ROX; 2 x 1.9 ml RNase-Free Water	221415
QIAprep&amp Viral RNA UM Kit (2400)	For 2400 x 20 µl reactions: 4 x1.2 ml Viral RNA UM Prep Buffer; 8 x 1.5 ml Viral RNA Master Mix, 4x; 4 x1.2 ml RNA IC Template + Assay; 4x0.6 ml Human Sampling IC Assay, 4 x 1 ml ROX; 8 x 1.9 ml RNase-Free Water	221417
SARS-CoV-2 N1+N2 Assay Kit (600)	For 600 x 20 µl reactions: 1x 600 µl SARS-CoV-2 N1+N2 assay, 20x concentrate	222015
SARS-CoV-2 N1+N2 Assay Kit (2400)	For 2400 x 20 µl reactions: 4x 600 µl SARS-CoV-2 N1+N2 assay, 20x concentrate	222017

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Document Revision History

Date	Changes
11/2020	Initial release

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