October 2020

Quick-Start Protocol

QIAprep&amp Viral RNA UM Kit

The QIAprep&amp Viral RNA UM Kit (cat. nos. 221415 and 221417) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The Viral RNA Master Mix and ROX Reference Dye can also be stored at 2–8°C for up to 12 months protected from light, depending on the expiration date.

Further information

- *QIAprep&amp Viral RNA UM Kit Handbook*: **www.qiagen.com/HB-2830**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

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®, Viroculit™, 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 Viral RNA UM Prep Buffer prepares the samples for the detection step but is not a virus inactivation solution.
- 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

QuantiNova 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.

- **Important**: Always start with the cycling conditions specified in this protocol.
- The PCR section of the RT-qPCR protocol must start with an initial incubation step of 2 min at 95°C to activate the DNA Polymerase.
- The RNA IC Template + Assay (Internal Control) 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 pre-mixed formulation (20x) contains forward and reverse primers and TaqMan probes.
- For viral targets, it is recommended to prepare a 20x primer–probe mix containing target-specific primers and probes. Viral sequences can be detected in the green channel on the Rotor-Gene Q or in the FAM dye channel on other real-time PCR instruments. We recommend to use 0.8 µM primers (forward/reverse) and 0.25 µM probe concentrations in the reaction. For further information, or to download the handbook or supplementary protocols, please visit the product page (**www.qiagen.com/qiaprepandamp-resources**).
- 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.

Procedure

1. Prepare a reaction mix according to Table 1 and mix thoroughly.
2. Vortex the swab containing the sample vigorously.
3. Dispense 2 µl of Viral RNA UM Prep Buffer into each PCR tube or a well of a PCR plate.
4. Add 8 µl of the sample to the same PCR tube or well containing the Viral RNA UM Prep Buffer. Mix by pipetting up and down at least twice.
5. Incubate at room temperature for 2 min.

   Note: Incubation time starts after adding the last sample to the Viral RNA UM Prep Buffer.
6. Add 10 µl of the reaction mix prepared in step 1.

Table 1. Reaction mix setup

Component	96/384-well block	Final concentration
Viral RNA Master Mix, 4x	5 µl	1x
20x primer–probe mix	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 (combined at step 6)	10 µl	–
Total reaction volume	**20 µl**	**–**

* To be used as a 20x concentrate for high-ROX dye cyclers (i.e., ABI PRISM 7000, Applied Biosystems 7300, 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).

7. **Important consideration**:

   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.

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

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

8. Program the real-time cycler according to Table 2.

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

Table 2. 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

* Annealing temperatures can be adapted between 55–62°C depending on primer/probe set used. For further details on cycling conditions, primer/probe concentrations, and annealing temperature, visit the product page (**www.qiagen.com/qiaprepandamp-resources**).

9. Place the tubes or plates in the real-time cycler and start the cycling program.
10. For results interpretation, refer to Table 3.

Table 3. 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)

Document Revision History

Date	Changes
10/2020	Initial release

Scan QR code for the product page and supplementary protocols.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.