Quick-Start Protocol November 2021

QIAprep&amp Viral RNA UM Kit

The QIAprep&amp Viral RNA UM Kit (cat. nos. 221413, 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®, Viocult™, 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 Quick-Start Protocol includes a recommended heat treatment step before the sample preparation step. 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 (refer to the kit handbook for instructions).
- **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 minutes at 95°C to activate the DNA Polymerase.

Procedure

1. 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.

2. Prepare a reaction mix according to Table 1 and mix thoroughly.
3. Vortex the swab containing the sample vigorously.
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. Dispense 2 µl of Viral RNA UM Prep Buffer into each PCR tube or a well of a PCR plate.
6. Transfer 8 µl of the sample to the same PCR tube or wells containing the Viral RNA UM Prep Buffer. Mix by pipetting up and down at least twice. 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.
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 with medium pressure. 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.

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).

9. Place the tubes or plates in the real-time cycler and perform cycling according to below conditions (Table 2).

   Program the real-time cycler before reaction setup 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**).

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
09/2020	Initial release
11/2021	Added the catalog number for the new kit size (100 rxn; cat. no. 221413). Incorporated the optional sample heat treatment in "Notes before starting" and "Procedure" sections. Removed several points in "Notes before starting".

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.