Quick-Start Protocol December 2021

Multitarget Detection Assay for Respiratory Viruses: SARS-CoV-2, Flu A, Flu B, RSV A/B

The multitarget assay is optimized to work with the QIAprep&amp™ Viral RNA UM Kit (**www.qiagen.com/HB-2830**) on human samples collected with nasal, nasopharyngeal, or oropharyngeal swabs stored in non-fixation transport media like UTM, VTM, PBS, ESwabs®, Virocult™, or 0.9% NaCl. Alternative starting materials such as saliva and gargle are suitable as well. The assay allows the detection of several respiratory viruses present in human samples in one reaction.

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

- **Important**: We recommend to use cycling conditions specified in this protocol.
- The assay can be performed on all common real-time PCR instrument with 5 channels (FAM, HEX, ROX, Cy5, and Cy5.5). For further details on setting adjustments for specific instruments, see our website (**www.qiagen.com/qiaprepamp-viral-rna-um-kit**).
- 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.
- Primers and probes (20x pre-mixed formulation) for the detection of each target are listed in Table 1 and delivered in separate tubes.
- In-process control (optional) is intended to report that the primary sample tube contains intact human genetic material and to ensure that the PCR reaction is not inhibited. The control will also make sure that no technical errors occurred during the experimental setup. The pre-mixed formulation (20x) contains forward and reverse primers and TaqMan® probes. The in-process control (RNAse P) is detected in the far red channel (Cy5.5/Crimson).

Procedure

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

Table 1. Reaction mix setup

Component	Channel for detection	20 µl rxn	25 µl rxn	50 µl rxn	Final concentration
Viral RNA Master Mix, 4x		5 µl	6.25 µl	12.5 µl	1x
Flu B assay*, 20x	FAM	1 µl	1.25 µl	2.5 µl	1x
Flu A assay*, 20x	HEX	1 µl	1.25 µl	2.5 µl	1x
SARS-CoV-2 N1/N2 assay*, 20x	ROX	1 µl	1.25 µl	2.5 µl	1x
RSV A/B assay, 20x	Cy5	1 µl	1.25 µl	2.5 µl	1x
In-process control, 20x	Cy5.5	1 µl	1.25 µl	2.5 µl	1x
Nuclease free water	-	-	2.5 µl	15 µl	-
Total volume		**10 µl**	**15 µl**	**40 µl**	**–**

* Primers and probes based on US-CDC design for the following targets: SARS-CoV-2 (Nucleocapsid gene/N1 and N2), Flu A (Matrix gene/M1), Flu B (Nonstructural 2 gene/NS2). RSV A/B (Matrix gene), QIAGEN own design

2. Vortex the sample vigorously.
3. Sample heat treatment:

   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

   3a. Recommended for swabs (70°C for 10 min).

   3b. Mandatory for saliva and gargle samples (95°C for 15 min). For additional information, please refer to the *QIAprep&amp Buffer AB Quick-Start Protocol*.
4. Centrifuge the plate/tube briefly.
5. Dispense 2 µl (for a 20 µl rxn) of the Viral RNA UM Prep Buffer into each PCR tube or well of a PCR plate.

6. Transfer 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 two times (if working with purified RNA, fill missing volume with, e.g., NaCl 0.9%). Incubate at room temperature for 2 min. This step can be excluded if working with purified RNA.

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

7. **Important consideration:**

   7a. 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, in order to obtain a tight seal across individual wells.

   7b. 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.

   7c. Centrifuge the plate/tube briefly to collect liquid at the bottom of the plate/tube. Place the tubes or plates in the real-time cycler and start the cycling program according to Table 2. A positive signal in the respective channel indicates the presence of the specified virus in the analyzed sample (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

Document Revision History

Date	Changes
12/2021	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.