August 2021

Quick-Start Protocol

SARS-CoV-2 LNA® qPCR Assays

SARS-CoV-2 LNA qPCR Assays are genotyping assays optimized to work with the QIAprep&amp™ Viral RNA UM Kit on human samples collected with nasal, nasopharyngeal, or oropharyngeal swabs stored in non-fixation transport media like UTM, VTM, PBS, or 0.9% NaCl. Alternative starting materials like saliva and gargle are suitable as well. The assays allow the detection of SARS CoV-2 mutations present in the variants of concern/variants of interest.

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 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 assays have been designed to run in singleplex. For further details and compatibility in duplex with other SARS-CoV-2 assays, see our website (**https://www.qiagen.com/applications/infectious-disease/coronavirus/research-solutions/covid-genotyping**).
- SARS-CoV-2 LNA qPCR Assays are FAM labeled and can be detected in the green channel on the Rotor-Gene Q or in the **FAM** dye channel on other real-time PCR instruments. The pre-mixed formulation (20x) contains forward and reverse primers and an LNA TaqMan® probe.

Procedure

1. Prepare a reaction mix according to Table 1.

Table 1. Reaction mix setup

Component	96/384-well block	Final concentration
Viral RNA Master Mix, 4x	5 µl	1x
SARS-CoV-2 LNA qPCR Assay, 20x (genotyping assay)	1 µl	1x
SARS-CoV-2 Assay (**optional**), 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, ViiA® 7, and QuantStudio® Real-Time PCR Systems).

2. Vortex vigorously the primary sample tube.

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 of 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 wells 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., 0.9% NaCl. Incubate at room temperature for 2 min. This step can be excluded if working with purified RNA.

   Note: The 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 (Table 1) to the same PCR tubes or wells.

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.

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

8. Place the tubes or plates in the real-time cycler and start the cycling program according to Table 2.

   Program the real-time cycler before the reaction setup according to Table 2.

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

9. A positive signal in the FAM channel indicates the presence of the specified mutation in the analyzed SARS-CoV-2 viral sample.

Document Revision History

Date	Changes
08/2021	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.