Quick-Start Protocol January 2021

QIAcuity® One-Step Viral RT-PCR Kit

This kit contains the following: 4x 1.3 ml One-Step Viral RT-PCR Master Mix (4x), 2x 100 µl Multiplex Reverse Transcription Mix (100x), and 8x 1.9 ml RNase-Free Water.

This protocol is optimized for the quantification of RNA targets using the QIAcuity One-Step Viral RT-PCR Kit with hydrolysis probes in a singleplex or multiplex (up to 5 targets) reaction using QIAGEN's QIAcuity instruments for digital PCR (dPCR).

The QIAcuity One-Step Viral RT-PCR Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The QIAcuity One-Step Viral RT-PCR Master Mix can also be stored protected from light at 2–8°C. Under these conditions, the components are stable for 12 months without showing any reduction in performance and quality, unless otherwise indicated on the label.

Further information

- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- *QIAcuity User Manual Extension*: **www.qiagen.com/HB-2839**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Refer to the *QIAcuity User Manual* and *QIAcuity User Manual Extension* for guidance on assay design and experimental setup for the QIAcuity platform.
- **Single- and multi-Nanoplate protocol**: Depending on the workflow, different protocols are provided to ensure optimal results. When processing just one plate at a time with any of the QIAcuity instruments, the single-Nanoplate protocol can be used.
  The single-Nanoplate protocol can also be used to process two plates

in parallel on the QIAcuity Eight instrument, as it contains two thermal cyclers that work in parallel. To process two or more plates in parallel on the QIAcuity Four instrument, or three or more plates in parallel on the QIAcuity Eight instrument, the multi-Nanoplate protocol is highly recommended.

Procedure

Reaction mix setup

1. Place the Multiplex Reverse Transcription Mix on ice. Thaw the QIAcuity One-Step Viral RT-PCR Master Mix, template RNA, primers, probes, and RNase-Free Water. Vigorously mix the QIAcuity One-Step Viral RT-PCR Master Mix and the individual solutions. Centrifuge the tubes briefly to settle the liquids.
2. Prepare a master mix according to Table 1 and the desired Nanoplate format.

Table 1. Preparing the QIAcuity One-Step Viral RT-PCR reaction mix

Component	Volume/reaction		
	Nanoplate 8.5k (96-well)	Nanoplate 26k (24-well)	Final concentration
4x One-Step Viral RT-PCR Master Mix	3 µl	10 µl	1x
100x Multiplex Reverse Transcription Mix	0.12	0.4 µl†	1x
20x primer–probe mix 1*	0.6 µl†	2 µl†	0.4 µM forward primer 0.4 µM reverse primer 0.2 µM probe
20x primer–probe mix 2, 3, 4, 5* (for multiplex)	0.6 µl†	2 µl†	0.4 µM forward primer 0.4 µM reverse primer 0.2 µM probe
RNase-Free Water	Variable	Variable	
Template RNA (added at step 5)†	Variable	Variable	
Total reaction volume	**12 µl**	**40 µl**	

* For dye recommendations, see *QIAcuity User Manual* or the *QIAcuity User Manual Extension*.

† Appropriate template amount depends on various parameters.

3. Vortex the reaction mix well. Dispense appropriate volumes of the reaction mix into the wells of a standard 96-well PCR pre-plate on ice.
4. Add template RNA to wells containing the reaction mix. To ensure mixing of the reaction mix with the template RNA, the pre-plate has to be sealed, shortly vortexed, briefly centrifuged, and then placed back on ice.

Multi-Nanoplate protocol for QIAcuity Four and Eight instruments

Note: The multi-Nanoplate protocol can also be used as an alternative protocol to the single-Nanoplate protocol for QIAcuity One instruments.

1. Program a thermal cycler with a heated lid according to Table 2.

Table 2. Reverse transcription program

Step	Time	Temperature
Reverse Transcription	30 min	50°C
RT Enzyme Inactivation	10 min	58°C
Infinite Hold	Hold	4°C

2. Place the PCR pre-plate from step 4 in the thermal cycler and start the reverse transcription program. Following the reverse transcription, transfer the contents of each well to the wells of a Nanoplate.
3. Seal the Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.
4. Program the cycler of the QIAcuity instrument according to Table 3.

Table 3. Multi-Nanoplate QIAcuity cycling program

Step	Time	Temperature
PCR initial heat activation	2 min	95°C
2-step cycling (40 cycles)		
Denaturation	5 s	95°C
Combined annealing/extension	30 s	60°C*

* Temperature during annealing/extension and number of cycles might vary depending on assay type.

5. Place the Nanoplate into the QIAcuity instrument and start the dPCR program.

Single-Nanoplate protocol for QIAcuity One

Note: If using this protocol on a QIAcuity Four or Eight instrument, the plate must be loaded onto an instrument with no plates queued ahead of it and run immediately after loading.

1. Program a QIAcuity instrument according to Table 4.

Table 4. Single-Nanoplate QIAcuity cycling program

Step	Time	Temperature
Reverse Transcription	40 min	50°C
PCR initial heat activation	2 min	95°C
2-step cycling (40 cycles)		
Denaturation	5 s	95°C
Combined annealing/extension	30 s	60°C*

* Temperature during annealing/extension and number of cycles might vary depending on assay type.

2. Transfer the contents of the standard PCR pre-plate to the wells of a Nanoplate.
3. Seal the Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.
4. Place the Nanoplate into the QIAcuity instrument and start the dPCR program.

Document Revision History

Date	Changes
01/2021	Initial release

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