

QIAcuity[®] OneStep Advanced Probe Kit

This protocol is optimized for the quantification of RNA and DNA targets using the QIAcuity OneStep Advanced Probe 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 OneStep Advanced Probe Kit should be stored immediately upon receipt at -30 to -15°C in a constant-temperature freezer and protected from light. 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.
- The QIAcuity OneStep Advanced Probe Kit has been specially formulated with a hot-start RT enzyme, allowing users to assemble reactions at room temperature and to run up to four and eight plates in parallel on the QIAcuity Four and QIAcuity Eight instruments, respectively.
- The optional Enhancer GC is recommended for use with all ABI TaqMan assays, amplicons >150 nt in length, GC rich amplicons, and RNA targets containing challenging secondary structures.

Procedure

Reaction mix setup

1. Place the 100x OneStep Advanced Reverse Transcription Mix on ice. Thaw the 4x QIAcuity One-Step Advanced Probe Master Mix, template RNA, primers, probes, Enhancer GC, and RNase-Free Water. Vigorously mix the QIAcuity One-Step Advanced Probe 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 Advanced Probe RT-dPCR reaction mix

Component	Volume/reaction		
	Nanoplate 8.5k (96-well)	Nanoplate 26k (24-well)	Final concentration
4x One-Step Advanced Probe Master Mix	3 μ l	10 μ l	1x
100x One-Step Advanced RT Mix (Reverse Transcription)	0.12	0.4 μ l	1x
20x primer–probe mix 1 *	0.6 μ l	2 μ l	0.4 μ M forward primer 0.4 μ M reverse primer 0.2 μ M probe
20x primer–probe mix 2, 3, 4, 5* (for multiplex)	0.6 μ l (each)	2 μ l (each)	0.4 μ M forward primer 0.4 μ M reverse primer 0.2 μ M probe
Enhancer GC [†] (optional)	1.5 μ l	5 μ l	
RNase-Free Water	Variable	Variable	
Template RNA (added at step 4) [‡]	Variable	Variable	
Total reaction volume	12 μl	40 μl	

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

[†] Enhancer GC is recommended for use with all ABI TaqMan assays, amplicons >150 nt in length, GC rich amplicons, and RNA targets containing challenging secondary structures.

[‡] 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.

Note: The pre-plate may be assembled at room temperature.

4. Add template RNA to wells containing the reaction mix. Thoroughly mix the template RNA with the reaction mix by pipetting up and down.

One-step RT-dPCR protocol for all QIAcuity instruments

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

Table 2. QIAcuity RT-dPCR cycling program

Step	Time	Temperature
Reverse Transcription	40 min	50°C
RT Enzyme Inactivation	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.

Ordering Information

Product	Contents	Cat. no.
QIAcuity OneStep Advanced Probe Kit (1 ml)	1 ml OneStep Advanced Probe Master Mix (4x), 45 µl OneStep RT Mix (100x), 1 ml Enhancer GC, 20 µl QN Internal Control RNA, 2 x 1.9 ml RNase-free water	250131
QIAcuity OneStep Advanced Probe Kit (5 ml)	5 x 1 ml OneStep Advanced Probe Master Mix (4x), 5 x 45 µl OneStep RT Mix (100x), 5 x 1 ml Enhancer GC, 1 x 20 µl QN Internal Control RNA, 8 x 1.9 ml RNase-free water	250132

Document Revision History

Date	Changes
03/2022	Initial release
04/2022	Corrected the step number referenced in Table 1.

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

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