March 2024

Quick-Start Protocol

QIAcuity® OneStep Advanced EvaGreen® Kit

This protocol is optimized for the quantification of RNA and DNA targets using the QIAcuity OneStep Advanced EvaGreen Kit (cat. nos. 250141 and 250142) in a singleplex reaction using QIAcuity instruments for digital PCR (dPCR).

The QIAcuity OneStep Advanced EvaGreen Kit should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer and protected from light. Under these conditions, kit performance will remain unaffected until the indicated date of expiration.

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 EvaGreen Kit has been specially formulated with a hot-start RT enzyme, allowing users to assemble reactions at room temperature (15–25°C), and to run up to four or eight plates in parallel on the QIAcuity Four (cat. no. 911042 or 911046) or QIAcuity Eight (cat. no. 911052 or 911056), respectively.

- Refer to the dedicated Quick-Start Protocol for using QuantiNova® LNA PCR Assays with the QIAcuity OneStep Advanced EvaGreen Kit.
- See handbook for guidance on using the QuantiNova Internal Control RNA.

Procedure

Reaction mix setup

1. Place the 100x OneStep Advanced Reverse Transcription Mix on ice. Thaw the 4x QIAcuity OneStep Advanced EvaGreen Master Mix, template RNA, primers, Q-Solution, and RNase-Free Water (cat. no. 129112). Vigorously mix the QIAcuity OneStep Advanced EvaGreen 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 OneStep Advanced EvaGreen RT-dPCR reaction mix

	Volume/reaction		
Component	**Nanoplate 8.5k (24-well and 96-well)**	**Nanoplate 26k (8-well and 24-well)**	**Final concentration**
4x OneStep Advanced EvaGreen Master Mix	3 µL	10 µL	1x
100x OneStep Advanced RT Mix (Reverse Transcription)	0.12 µL	0.4 µL	1x
20x primer mix	0.6 µL	2 µL	0.75 µM forward primer 0.75 µM reverse primer
Q-Solution*	1 µL	3.3 µL	–
RNase-Free Water	Variable	Variable	–
Template RNA (added at step 4)†	Variable	Variable	–
Total reaction volume	**12 µL**	**40 µL**	

* The use of Q-Solution is strongly recommended, but the PCR reaction will work if users choose to omit it. Q-solution is especially beneficial for use with amplicons >150 bp in length, GC-rich amplicons, and RNA targets containing challenging secondary structures. The reaction mix may become faintly cloudy when Q-solution is added. However, this has no impact on PCR performance and should largely resolve when the reaction mix reaches its final 1x concentration.

† 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.

RT-dPCR protocol for all QIAcuity instruments

5. Transfer the contents of each well in the pre-plate to the wells of a Nanoplate.
6. Seal the Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.
7. Place the Nanoplate into the QIAcuity instrument and start the RT-dPCR program. Recommended cycling conditions for two-step and three-step RT-dPCR are described in Table 2 and Table 3. Adjust the Imaging Settings according to Table 4.

Table 2. Two-step QIAcuity RT-dPCR cycling program

Step	Time	Temperature (°C)*
Reverse Transcription	40 min	50
RT Enzyme Inactivation	2 min	95
2-step cycling (40 cycles)	–	–
Denaturation	10 s	95
Combined annealing/extension	30 s	55–60
Cooling Down	5 min	40

* Temperature during annealing/extension and number of cycles might vary depending on assay type. As a starting point, we recommend that users first begin with 40 cycles and an annealing/extension temperature of 58°C.

Table 3. Three-step QIAcuity RT-dPCR cycling program

Step	Time	Temperature (°C)*
Reverse Transcription	40 min	50
RT Enzyme Inactivation	2 min	95
3-step cycling (40 cycles)	–	–
Denaturation	15 s	95
Annealing	15 s	55–60
Extension	15 s	72
Cooling Down	5 min	40

* Temperature during annealing and number of cycles might vary depending on assay type. As a starting point, we recommend that users first begin with 40 cycles and an annealing temperature of 58°C.

Table 4. Imaging Settings

Channel	Exposure Duration	Gain
Green	200 ms	3

Document Revision History

Date	Changes
September 2023	Initial release.
March 2024	Removed QuantiTect Primer Assays and RT² qPCR Assays references from "Notes before starting"

Scan QR code for the *QIAcuity User Manual*.

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