



September 2023

Quick-Start Protocol

## RT<sup>2</sup> qPCR Primer Assays with the QIAcuity<sup>®</sup> OneStep Advanced EvaGreen<sup>®</sup> Kit

This protocol is optimized for the quantification of mRNA targets using the RT<sup>2</sup> qPCR Primer Assays (cat. nos. 330001) with the QIAcuity OneStep Advanced EvaGreen Kit (cat. nos. 250141 and 250142) in a singleplex reaction using QIAcuity instruments for digital PCR (dPCR).

The RT<sup>2</sup> qPCR Primer Assays are shipped at ambient temperature but must be stored at –30 to –15°C upon arrival. Under these conditions, the components are stable for 12 months without showing any reduction in performance and quality, unless otherwise indicated on the label.

The QIAcuity OneStep Advanced EvaGreen Kit should be stored immediately upon receipt at –30 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](http://www.qiagen.com/HB-2717)
- *QIAcuity User Manual Extension*: [www.qiagen.com/HB-2839](http://www.qiagen.com/HB-2839)
- Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety)
- Technical assistance: [support.qiagen.com](http://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, 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.
- The QuantiNova® Internal Control RNA (QN IC RNA) supplied with the kit can be used optionally as a reverse transcription and amplification control. To do so, users must separately purchase the Ctrl\_QNIC\_1\_SG QuantiTect® Primer Assay (QT02589307) and use it a final concentration of 1x.
- The QN IC RNA comes at a concentration of  $\sim 1 \times 10^5$  to  $1 \times 10^6$  copies/ $\mu\text{L}$ . Users are recommended to add the QN IC RNA to their QIAcuity reactions at a final dilution of 1:1000 (e.g. create a working dilution by diluting the QN IC RNA stock 1:10 in RNase-Free Water (cat. no. 129112), which is then diluted 1:100 in the QIAcuity OneStep Advanced EvaGreen reaction mix.)

## 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. 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**

Component	Volume/reaction		
	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
RT <sup>2</sup> qPCR Primer Assay (10 µM stock)	0.9 µL	3 µL	0.75 µM
Q-Solution <sup>†</sup>	1 µL	3.3 µL	–
RNase-Free Water	Variable	Variable	–
Template RNA (added at step 4) <sup>‡</sup>	Variable	Variable	–
<b>Total reaction volume</b>	<b>12 µL</b>	<b>40 µL</b>	

<sup>†</sup> Adding Q-Solution to PCR reactions is strongly recommended for all users, but the PCR reaction will work if it is omitted. 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.

<sup>‡</sup> 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 (cat. no. 250031).\*
3. Place the Nanoplate into the QIAcuity instrument and start the RT-dPCR program described in Table 2.

\* Other options are available at [www.qiagen.com](http://www.qiagen.com)

4. Imaging should be performed in the “Green channel” using an exposure duration of 200 ms and a gain setting of 3.

**Table 2. QIAcuity RT-dPCR cycling program for RT<sup>2</sup> qPCR Primer Assays**

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

## Document Revision History

Date	Changes
September 2023	Initial release



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.

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