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Quick-Start Protocol

QuantiNova® LNA® PCR Assays with the QIAcuity® EG PCR Kit

This protocol is optimized for the quantification of mRNA/lncRNA targets (cDNA) using the QuantiNova LNA PCR Assays (cat. nos. 249990 and 249992) with the QIAcuity EG PCR Kit (cat. nos. 250111, 250112, and 250113), using the QIAcuity digital PCR (dPCR) instrument. For detection, EvaGreen® is used as an intercalating dye in the dPCR reaction.

The QuantiNova LNA PCR Assays are shipped at room temperature (15–25°C). Upon receipt, store QuantiNova LNA PCR Assays at 2–8°C for short-term storage or at –30 to –15°C in a constant-temperature freezer for long-term storage. After reconstitution, store PCR assays in aliquots at –30 to –15°C to avoid repeated freeze–thaw cycles. Unless otherwise indicated on the label, the components are stable for 12 months without showing any reduction in performance under these conditions.

The QIAcuity EG PCR Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The QIAcuity EG PCR master mix can also be stored protected from light at 2–8°C. Unless otherwise indicated on the label, the components are stable for 12 months without showing any reduction in performance under these conditions.

Further information

- *QIAcuity User Manual Extension: Application Guide*: **www.qiagen.com/HB-2839**
- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- *QuantiNova LNA PCR Handbook for the QIAcuity System*: **www.qiagen.com/HB-2813**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- A fluorescent reference dye is provided as a component of the QIAcuity EG PCR master mix for reliable detection of proper partition filling in the dPCR Nanoplates.
- Resuspend the QuantiNova LNA PCR Assay: Centrifuge the tube before opening it for the first time. Add 440 µl nuclease-free water (for QuantiNova LNA PCR Assay for 200 reactions) or 1650 µl nuclease-free water (for QuantiNova LNA PCR Assay for 750 reactions) to the tube and leave at room temperature for 20 min. Vortex and briefly centrifuge. The stock concentration of the reconstituted QuantiNova LNA PCR Assays is 10x. In the dPCR reaction using the QIAcuity EG PCR Kit, half of the assay concentration is used; therefore, the final assay concentration in the reaction setup is 0.5x.
- Always start with the cycling conditions and primer concentrations specified in this protocol.
- For 2-step RT-PCR, use the QuantiTect® Reverse Transcription Kit for the first step to synthesize the cDNA.

  Important: The QuantiNova Reverse Transcription Kit is not recommended.

  The volume of the cDNA added (from the undiluted reverse-transcription reaction) should not exceed 10% of the final PCR volume if using the QuantiTect Reverse Transcription Kit. If using another RT Kit, it should not exceed 5%.

Procedure

Reaction setup

1. Thaw the QIAcuity EG PCR master mix, template cDNA, 10x QuantiNova LNA PCR Assay, and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 1. Due to the hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity dPCR instrument.
3. Vortex the reaction mix.
4. Dispense appropriate volumes of the reaction mix, which contains all components except the template, into the wells of a standard PCR plate. Then, add template cDNA into each well that contains the reaction mix.

Note: The appropriate amounts of reaction mix and template DNA depends on various parameters. Please refer to the *QIAcuity User Manual Extension: Application Guide* for details.

Table 1. Reaction setup

Component	Volume/reaction		
	Nanoplate 8.5k (24-well, 96-well)	**Nanoplate 26k (24-well)**	**Final concentration**
3x EvaGreen PCR Master Mix (green channel)	4 µl	13.3 µl	1x
QuantiNova LNA PCR Assay (10x)	0.6 µl	2 µl	**0.5x***
RNase-free water	Variable	Variable	
Template cDNA (added at step 4)	Variable†	Variable†	≤15% of total reaction volume
Total reaction volume	**12 µl**	**40 µl**	

* Assay concentration in dPCR is half in comparison to qPCR use; therefore, the final concentration is 0.5x.

† Appropriate template amount depends on various parameters. For detailed information, please refer to the *QIAcuity User Manual Extension: Application Guide*.

5. Transfer the contents of each well of the standard PCR plate to the wells of the QIAcuity Nanoplate.
6. Seal the QIAcuity Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits. For the exact sealing procedure, please refer to the *QIAcuity User Manual*.

Thermal cycling and imaging conditions

1. In the QIAcuity Software Suite or on the QIAcuity instrument, under the dPCR parameters, set the cycling conditions according to Table 2.

Table 2. Cycling conditions

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
3-step cycling (40 cycles)		
Denaturation	15 s	95
Annealing	15 s	55
Extension	15 s	72
Cooling down	5 min	40

2. Under the dPCR parameters in the QIAcuity Software Suite or on the QIAcuity instrument, activate the green channel and deactivate the other channels in **Imaging**.
3. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

Data analysis

1. To set up a plate layout according to the experimental design, open the QIAcuity Software Suite and define the reaction mixes, samples, and controls. Plate layout can be defined before or after the nanoplate run.

   Note: Refer to the *QIAcuity User Manual* for details on setting up the plate layout.
2. After the run is completed, the raw data are automatically sent to the QIAcuity Software Suite.
3. For data analysis, open the QIAcuity Software Suite and select the individual nanoplate for the analysis in **Plate Overview** of the software suite.

   Note: See the *QIAcuity User Manual Extension: Application Guide* and *QIAcuity User Manual* for details on how to analyze absolute quantification data and gene expression data including normalization.

Document Revision History

Date	Changes
07/2020	Initial release
01/2021	Updated the URL for *QIAcuity User Manual Extension: Application Guide*. Removed the note regarding the use of the QuantiTect Reverse Transcription Kit for the first step to synthesize the cDNA.

Scan QR code for the *QIAcuity User Manual Extension: Application Guide*.

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