September 2021

Quick-Start Protocol

miRCURY® LNA® miRNA PCR Assays with the QIAcuity® EG PCR Kit

This protocol is optimized for the quantification of miRNAs using the miRCURY LNA miRNA PCR Assays (cat. nos. 339306 and 339317) with the miRCURY LNA RT Kit (cat. no. 339340) and 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 miRCURY LNA miRNA PCR Assays are shipped lyophilized at room temperature. They should be stored immediately upon receipt at –30 to 4°C. After resuspension of primer sets, it is recommended to store them in aliquots at –30 to –15°C to avoid repeated freeze–thaw cycles.

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

- *miRCURY LNA PCR Assay Handbook for the QIAcuity System*: **www.qiagen.com/HB-2947**
- *miRCURY LNA RT Kit Quick-Start Protocol:* **www.qiagen.com/HB-2424**
- *QIAcuity User Manual:* **www.qiagen.com/HB-2717**
- *QIAcuity User Manual Extension:* Application Guide: **www.qiagen.com/2839**

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for detection of miRNA targets via dPCR. For detection with any real-time cycler, please refer to the *miRCURY LNA miRNA PCR Handbook.*
- 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 miRCURY LNA PCR Assay: Spin down the tube before opening for the first time. Add 220 µl nuclease-free water and leave at room temperature for 20 min. Vortex and spin down briefly. The stock concentration of the reconstituted miRCURY LNA PCR Assay is 10x and is used with a final assay concentration of 1x in the reaction setup.
- Always start with the cycling conditions and primer concentrations specified in this protocol.

Procedure

Reaction setup

1. Thaw the QIAcuity EG PCR Master Mix, template cDNA, miRCURY LNA PCR Assay, and RNase-free water. Mix the individual solutions.
2. Perform dilutions of the cDNA. Dilute the cDNA 60x by adding 590 µl RNase-free water per 10 µl RT-reaction immediately before use.

   Note: When working with serum/plasma samples, the cDNA is diluted 30x.
3. Prepare a reaction mix according to Table 1. Due to the hot-start of the PCR reactions, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity dPCR instrument.
4. Vortex the reaction mix.
5. Dispense appropriate volumes of the reaction mix into the wells of a standard PCR plate.

Note: The appropriate amount of reaction mix and template DNA depends on various parameters. Please refer to *the miRCURY LNA PCR Assay Handbook for the QIAcuity System* and to the *QIAcuity User Manual Extension: Application Guide* for details.

Table 1. PCR reaction setup

Component	Nanoplate 8.5k (24-well, 96-well)	Nanoplate 26k (24-well)
3x EvaGreen PCR Master Mix (green channel)	4 µl	13.3 µL
miRCURY LNA PCR Assay, 10x	1.2 µl	4 µL
RNase-free water	3.8 µl*	12.7 µl*
Template cDNA	3 µl*	10 µl*
Total reaction volume	**12 µl**	**40 µl**

* Appropriate template amount depends on various parameters. For detailed information, please refer to the *miRCURY LNA PCR Handbook for the QIAcuity System* as well as to the *QIAcuity User Manual Extension: Application Guide*.

6. Transfer the contents of each well of the standard PCR plate to the wells of the QIAcuity Nanoplate.
7. 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.
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.

Table 2. Cycling conditions

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
2-step cycling (40 cycles)		
Denaturation	15 s	95
Annealing/Extension	1 min	60
Cooling down	5 min	40

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 QIAcuity Software Suite.

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

Document Revision History

Date	Changes
09/2021	Initial release

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