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Supplementary Protocol

## Detection of miRNAs using miRCURY<sup>®</sup> LNA<sup>®</sup> miRNA PCR Panels on a QIAcuity<sup>®</sup> Digital PCR System

This document describes the usage of miRCURY LNA miRNA PCR panels such as miRCURY LNA miRNA miRNome Panels (cat. no. 339322), miRCURY LNA miRNA Focus PCR Panels (cat. no. 339325), and fully customized miRCURY LNA miRNA Custom PCR panels (cat. no. 339330) for sensitive detection of miRNAs on a QIAcuity system in combination with the QIAcuity EG PCR Kit.

**Important:** Please refer to the *miRCURY LNA miRNA PCR Assay Handbook for the QIAcuity System* for general information on handling and storage of kit components as well as for detailed information about the experimental setup of RT and dPCR reaction.

### Equipment and reagents to be supplied by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- miRCURY LNA RT Kit (cat. no. 339340) and QIAcuity EG PCR Kit (cat. nos. 250111, 250112, and 250113)
- QIAcuity Nanoplates (cat. nos. 250001, 250011, and 250021)
- Sealing foils for dPCR plates (included with the Nanoplate kits, or sold separately as cat. no. 250099)
- High-quality nuclease-free water (do not use DEPC-treated water)
- Nuclease-free plastic tubes for 20 µl reactions
- Nuclease-free PCR tubes or plates for use with individual assays
- Nuclease-free, aerosol-barrier pipette tips
- Single-channel or multi-channel pipettor
- PCR cycler, heating block, or water bath (capable of reaching 95°C)
- Vortexer
- Microcentrifuge and plate centrifuge

## Things to do before starting (dPCR)

- Perform a cDNA synthesis according to the corresponding protocols provided in *miRCURY LNA miRNA PCR Assay Handbook for the QIAcuity System*.
- Thaw the 3x QIAcuity EG PCR Master Mix, template cDNA, miRNA PCR panel(s), and nuclease-free water. Vortex and spin down briefly.

## Procedure

1. 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.
2. Vortex the reaction mix thoroughly, and dispense 12 µl per well into the miRCURY PCR panel. The panel serves as pre-plate for the dPCR.

**Note:** The experiment can be paused at this point. Store the reactions protected from light at 2–8°C for up to 24 h.

3. Seal the plate. Carefully vortex it to dissolve the primers. Spin down briefly and incubate at 40°C for 5 min (e.g., in a PCR cycler).
4. Cool down the reaction for 10 min at room temperature.
5. Vortex and spin down briefly.
6. Transfer 11.5 µl of the reaction mix from each well of the PCR pre-plate to the wells of a 8.5k Nanoplate.

**Important:** Prevent introducing air bubbles during the transfer.

7. Seal the QIAcuity Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.

**Note:** For exact sealing procedure, please see the *QIAcuity User Manual* ([www.qiagen.com/HB-2717](http://www.qiagen.com/HB-2717)).

**Table 1. Reaction mix setup for miRCURY LNA miRNA PCR assays**

Component	Setup for 1 reaction (µl)	Setup for 96-well panel (µl) 105 x 12 µl reactions
3x EvaGreen® PCR Master Mix (green channel)	4	420
Template cDNA	Variable*	Variable*
RNase-free water	Variable*	Variable*
<b>Total reaction volume</b>	<b>12</b>	<b>1260</b>

\* Appropriate template amount depends on various parameters. For detailed information, please refer to the *miRCURY LNA miRNA PCR Handbook* or to the *miRCURY LNA miRNA PCR – Exosomes, Serum/Plasma and Other Biofluid Samples Handbook*.

## 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. Still 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 QIAcuity Nanoplate into the QIAcuity instrument and start the dPCR run.

**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
10/2021	Initial revision

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