Quick-Start Protocol September 2019

miRCURY® LNA® miRNA Probe PCR Assays and PCR Panels

The miRCURY Probe PCR Kits (cat. nos. 339371, 339372, and 339373) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The miRCURY Probe PCR Kits can also be stored protected from light at 2–8°C for up to 12 months, depending on the expiration date. The miRCURY LNA miRNA Probe PCR Assays and PCR Panels are shipped dried down at room temperature. Immediately upon receipt, they should be stored protected from light at 2 to 8°C for short-term storage or at –30 to –15°C for long-term storage. After resuspension of Probe Assays, it is recommended to store them protected from light in aliquots at –30 to –15°C to avoid repeated freeze-thaw cycles.

Further information

- *miRCURY LNA miRNA Probe PCR Handbook*: **www.qiagen.com/HB-2624**
- *miRCURY LNA miRNA Probe PCR – Exosomes, Serum/Plasma, and Other Biofluid Samples Handbook*: **www.qiagen.com/HB-2623**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for the detection of microRNA targets with any real-time cycler and conditions for fluorescence normalization.
- If using the Rotor-Gene Q, please see protocol details from the *miRCURY LNA miRNA Probe PCR Handbook*.

- ROX™ dye is required at the following concentrations:

  No requirement for ROX dye: Rotor-Gene®, Bio-Rad® CFX, Roche® LightCycler® 480, and Agilent® Technologies Mx instruments.
  Low concentration of ROX dye: Applied Biosystems® 7500, ViiA®7, and QuantStudio™ Real-Time PCR Systems.
  High concentration of ROX dye: ABI PRISM® 7000, Applied Biosystems 7300 and 7900, and StepOne™ Real-Time PCR Systems.
- The ROX Reference Dye should be used as a 20x concentrated solution for a 1x reaction when using an instrument requiring a high ROX dye concentration. For instruments requiring a low ROX dye concentration, use the dye as a 200x concentrate.
- The 2x QuantiNova® Probe PCR Master Mix contains QuantiNova DNA Polymerase, which is inactive at room temperature.
- The PCR protocol must start with an initial incubation step of 2 min at 95°C to activate the QuantiNova DNA Polymerase.
- Always start with the cycling conditions and primer concentrations specified in this protocol.

1. If using single assays, prepare the miRCURY LNA miRNA Probe PCR Assay: spin down the tube before opening for the first time. Add 220 µl nuclease-free water and leave for 20 min. Vortex and spin down.
2. Thaw Master Mix, template cDNA, Probe PCR Assays or Probe PCR Panels, ROX Reference Dye (if required), and RNase-free water. Mix the individual solutions.
3. Perform dilutions of the cDNA. If working with biofluids, please refer to the dilutions described in the dedicated handbook.

   For Probe Assays, dilute the cDNA 1:40 by adding 390 µl RNase-free water per 10 µl RT-reaction immediately before use.
   For Custom Probe PCR Panels (plates), dilute the cDNA 1:60 by adding RNase-free water immediately before use. For more information about the dilutions for each Custom Probe PCR Panel format, please refer to the handbook.
   For the other formats, there is no need to dilute the cDNA.

4. 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 real-time cycler.

Table 1. Reaction mix setup

Component	miRCURY LNA miRNA Probe PCR Assay	miRCURY LNA miRNome Probe PCR Panel (Panel I)*	miRCURY LNA miRNA Custom Probe PCR Panel†
2x QuantiNova Probe Master Mix	5 µl	2000 µl	5 µl
10x miRCURY Probe Universal Primer	1 µl	400 µl	1 µl
ROX Reference Dye (AB instruments only)	0.5 µl/0.05 µl‡	200 µl/20 µl‡	0.5 µl/0.05 µl‡
Resuspend Probe Assay	1 µl	-	-
cDNA template	2 µl (diluted 1:40)	20 µl	3 µl (diluted 1:60)
RNase-free water	1 µl‡	1580 µl‡	1 µl‡
Total reaction volume§	**10 µl**	**4000 µl**	**10 µl**

* The reaction volume shown is per 384-well plate.

† The volume shown is for a single reaction. Depending on plate layout, calculate the required reaction volume. See handbook for more information.

‡ To be used as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300 and 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA7, and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

§ The reaction volume for Rotor-Gene Q is 20 µl. Please see the *miRCURY LNA miRNA Probe PCR Handbook* for protocol details.

5. Mix the reaction thoroughly and dispense 10 µl from each well into PCR tubes or plates.
6. Spin the plate briefly in a cooled centrifuge. If using panels, wait 5 min for the Probe Assays to dissolve.
7. Program the real-time cycler according to Table 2.

   Note: Data acquisition should be performed during the annealing/extension step.
8. Place the PCR tubes or plates in the real-time cycler and start the cycling program.

Table 2. Cycling conditions

Step	Time	Temperature	Ramp rate	Additional comments
PCR initial heat activation	2 min	95°C	Maximal/fast mode	
2-step cycling				
Denaturation	5 s	95°C	Maximal/fast mode	
Combined annealing/extension	30 s	56°C	Maximal/fast mode	Perform fluorescence data collection (FAM-Dark Quencher)
Number of cycles	40*			

* If using a Roche LightCycler 480, use 45 cycles.

9. For interpreting the results, please refer to the *miRCURY LNA miRNA Probe PCR Handbook*.

Document Revision History

Date	Changes
09/2019	Initial release

Scan QR code for *miRCURY LNA miRNA Probe PCR Handbook*.

Scan QR code for *miRCURY LNA miRNA Probe PCR – Exosomes, Serum/Plasma, and Other Biofluid Samples Handbook*.

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