Quick-Start Protocol September 2017

miRCURY® LNA® miRNA PCR Assays and PCR Panels

The miRCURY LNA SYBR® Green PCR Kit (cat. nos. 339345, 339346, 339347) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The miRCURY LNA SYBR Green PCR Kit 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 PCR Assays and PCR Panels are shipped dried down at room temperature. They should be stored immediately upon receipt at –30°C 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.

Further information

- *miRCURY LNA miRNA PCR Handbook*: **www.qiagen.com/HB-2431**
- *miRCURY LNA miRNA PCR – Exosomes, Serum/Plasma and Other Biofluid Samples Handbook*: **www.qiagen.com/HB-2439**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for detection of microRNA targets with any real-time cycler and conditions for fluorescence normalization. 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, 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 miRCURY LNA SYBR Green 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 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, primer sets or PCR panels, ROX Reference Dye (if required) and RNase-free water. Mix the individual solutions.
3. Perform dilutions of the cDNA.

   For primer sets, dilute the cDNA 60x by adding 1180 µl RNase-free water per 20 µl RT-reaction immediately before use.

   For Custom PCR Panels (plates), dilute the cDNA 80x by adding 1580 µl RNase-free water per 20 µl RT-reaction immediately before use.

   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 PCR Assay	miRCURY LNA miRNome PCR Panels – human, mouse, and rat (Panel I+II)	miRCURY LNA Focus PCR Panel – serum/Plasma	miRCURY LNA Focus PCR Panel – cancer	Pick-&-Mix
2x miRCURY SYBR Green Master Mix	5 µl	2000 µl	1000 µl	500 µl	5 µl
ROX Reference Dye (AB instruments only)	0.5 µl/0.05 µl*	200 µl/20 µl*	100 µl/10 µl*	50 µl/5 µl*	0.5 µl/0.05 µl*
PCR primer mix	1 µl	-	-	-	-
cDNA template	3 µl (60x diluted)	20 µl	20 µl	5 µl	4 µl (80x diluted)
RNase-free water	1 µl	1980 µl*	980 µl*	495 µl*	1 µl
Total reaction volume	**10 µl**	**4000 µl**	**2000 µl**	**1000 µl**	**10 µl**

* To be used as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 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 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.

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 primers 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
PCR initial heat activation	2 min	95°C	Maximal/fast mode
2-step cycling			
Denaturation	10 s	95°C	Maximal/fast mode
Combined annealing/extension	60 s	56°C	Maximal/fast mode
Number of cycles	40*		
Melting curve analysis	60–95°C		

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

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

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

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