Quick-Start Protocol March 2020

QuantiNova® LNA® Probe PCR Panels

The QuantiNova LNA Probe PCR Panels (cat. nos. 249955, 249956, 249965, and 249975) are shipped 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 in constant-temperature freezer for long-term storage. Under these conditions, all components are stable for at least 12 months if not otherwise indicated on the label.

Note: All QuantiNova LNA Probe PCR Panels should be protected from light and stored in the dark.

Further information

- *QuantiNova LNA Probe PCR Handbook*: **www.qiagen.com/HB-2699**
- Product Data Sheets, including plate assay layout for QuantiNova LNA Probe PCR Focus Panels and QuantiNova LNA Probe PCR lncRNA Focus Panels: **www.qiagen.com**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for the detection of mRNA or lncRNA 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 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.

Procedure:

The QuantiNova LNA Probe PCR Panels can used in two different protocols: the 2-Step RT-PCR Protocol, which will be described below, and the 1-Step RT-PCR Protocol with one combined cDNA RT and the PCR reaction. For the 1-step RT-PCR Protocols, please refer to the *QuantiNova LNA Probe PCR Handbook.*

2-Step RT-PCR procedure:

1. Thaw 2x QuantiNova Probe PCR Master Mix, template cDNA, QN ROX Reference Dye (if required), and RNase-free water. Mix the individual solutions.
2. When using the QuantiNova Reverse Transcription Kit, add 90 µl RNase-free water to each 20 µl reverse transcription reaction to dilute the cDNA. Mix by pipetting up and down several times.
3. Prepare a Master Mix for 1 sample according to Table 1 or for more than 1 sample according to Table 2 or Table 3. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.
   Note: Save the remaining volume of the cDNA synthesis reaction at –15 to –30°C, for potential quality control analysis.

Table 1. Master Mix setup for QuantiNova LNA Probe PCR Panels (Focus, Flexible or Custom) for 1 sample

Component	96-well panels	384-well panels	Final concentration
2x QuantiNova Probe PCR Master Mix	1000 µl	2000 µl	1 x
ROX Reference Dye (ABI instruments only)	100 µl/ 10 µl*	200 µl/ 20 µl*	1 x
Diluted cDNA template	100 µl	100 µl	-
RNase-free water	Variable	Variable	-
Total Master Mix volume	**2000 µl†**	**4000 µl†**	**-**

* Use ROX Reference Dye 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, ViiA 7, and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Total Master Mix volume includes a reserve to compensate for pipetting variations

Table 2. Master Mix setup for QuantiNova LNA Probe PCR Flexible Panels and Custom Panels for more than 1 sample per 96-well plate/Rotor-Disc® 100

Component	2 samples (48 wells per sample)	4 samples (24 wells per sample)	8 samples (12 wells per sample)	Final concentration
2x QuantiNova Probe PCR Master Mix	520 µl	280 µl	160 µl	1x
ROX Reference Dye (ABI instruments only)	52 µl/5.2 µl*	28 µl/2.8 µl*	16 µl/1.6 µl*	1x
Diluted cDNA template	100 µl	100 µl	100 µl	–
RNase-free water	Variable	Variable	Variable	–
Total Master Mix volume	**2x 1040 µl†**	**4x 560 µl†**	**8x 320 µl†**	**–**

* Use ROX Reference Dye 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, ViiA 7 and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Each Master Mix includes a reserve of at least 4 single reaction volumes (80 µl for 96-well plates and 40 µl for 384-well plates) to compensate for pipetting variations.

Table 3. Master Mix setup for QuantiNova LNA Probe PCR flexible panels and custom panels for more than 1 sample per 384-well plate

Component	2 samples (192 wells per sample)	4 samples (96 wells per sample)	8 samples (48 wells per sample)	16 samples (24 wells per sample)	Final concentration
2x QuantiNova Probe PCR Master Mix	1000 µl	500 µl	260 µl	140 µl	1x
ROX Reference Dye (ABI instruments only)	100 µl/10 µl*	50 µl/5 µl*	26 µl/2.6 µl*	14 µl/1.4 µl*	1x
Diluted cDNA template	100 µl	100 µl	100 µl	100 µl	-
RNase-free water	Variable	Variable	Variable	Variable	-
Total Master Mix volume	**2x 2000 µl†**	**4x 1000 µl†**	**8x 520 µl†**	**16x 280 µl†**	**-**

* Use ROX Reference Dye 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, ViiA 7 and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Each Master Mix includes a reserve of at least 4 single reaction volumes (80 µl for 96-well plates and 40 µl for 384-well plates) to compensate for pipetting variations.

4. Mix the reaction mix thoroughly and dispense 20 µl per well (for 96-well formats) or 10 µl per well (for 384-well formats) into the PCR plates.

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

5. Seal the plates. Carefully vortex it to dissolve the primers (optional). Briefly centrifuge the plates at room temperature. Wait 5 min while the primers dissolve in the reaction mix.
6. Program the real-time cycler according to Table 4.

   Note: Data acquisition should be performed during the annealing/extension step.
7. Place the plates into the real-time cycler and start the cycling program.

Table 4. Cycling conditions

Step	Time	Temperature	Ramp rate	Additional comments
PCR initial heat activation	2 min	95°C	Maximal/fast mode	QuantiNova DNA Polymerase is activated by this heating step
2-step cycling				
Denaturation	5 s	95°C	Maximal/fast mode	
Combined annealing/extension	5 s*	60°C	Maximal/fast mode	Perform fluorescence data collection (FAM-Dark Quencher)
Number of cycles	45*			

* If your cycler does not accept this short time for data acquisition, choose the shortest acceptable time (e.g., 31 s annealing/extension for the ABI PRISM 7000 or Applied Biosystems 7300).

8. For interpreting the results, please refer to the *QuantiNova LNA Probe PCR Handbook*.

Document Revision History

Date	Changes
03/2020	Initial release

Scan QR code for *QuantiNova LNA Probe PCR Handbook*.

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