Quick-Start Protocol March 2020

QuantiNova® LNA® PCR Panels

The QuantiNova LNA PCR Panels (cat. nos. 249950, 249951, 249960, and 249970) are shipped at room temperature. Immediately upon receipt, they should be stored 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.

Further information

- *QuantiNova LNA PCR Handbook:* **www.qiagen.com/HB-2698**
- Product Data Sheets, including plate assay layout for QuantiNova LNA PCR Focus Panels and QuantiNova LNA 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/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, 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 SYBR® Green PCR Master Mix contains the 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 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 PCR Handbook.*

2-Step RT-PCR procedure:

1. Thaw 2x QuantiNova SYBR® Green 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 PCR Panels for 1 sample

Component	96-well panels	384-well panels	Final concentration
2x QuantiNova SYBR® Green PCR Master Mix	1000 µl	2000 µl	1x
ROX Reference Dye (ABI instruments only)	100 µl/10 µl*	200 µl/20 µl*	1x
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 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 SYBR® Green 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	2 x 1040 µl†	4 x 560 µl†	8 x 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 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 SYBR® Green 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	2 x 2000 µl†	4 x 1000 µl†	8 x 520 µl†	16 x 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. PCR cycling conditions for QuantiNova LNA PCR Panels

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	10 s*	60°C	Maximal/fast mode	Perform fluorescence data collection
Number of cycles	45			
Melting curve analysis†				

* 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).

† Melting curve analysis is an analysis step built into the software of real-time cyclers. To perform the analysis, follow instructions provided by the supplier

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

Document Revision History

Date	Changes
03/2020	Initial release

Scan QR code for *QuantiNova LNA PCR Handbook*.

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