Quick-Start Protocol March 2016

QuantiNova™ Probe PCR Kit

The QuantiNova Probe PCR Kit (cat. nos. 208252, 208254, 208256) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. QuantiNova Probe PCR Master Mix can also be stored protected from light at 2–8°C for up to 6 months, depending on the expiration date.

Further information

- *QuantiNova Probe PCR Handbook*: **www.qiagen.com/HB-1593**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for quantification of gDNA or cDNA targets using TaqMan® probes in a singleplex or duplex reaction with any real-time cycler and condition for fluorescence normalization. ROX™ dye is required for various cyclers 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, and ViiA™7 Real-Time PCR Systems

  High concentration of ROX dye: Applied Biosystems 7000, 7300, 7900 and StepOne™ Real-Time PCR Systems
- QN ROX Reference Dye is provided as a separate tube of passive reference dye for normalization of fluorescent signals on all real-time cyclers from Applied Biosystems. It should be diluted 1:20 in the 1x reaction when used on instruments requiring a high ROX concentration and 1:200 for instruments requiring a low ROX concentration.

- The dye in QuantiNova Yellow Template Dilution Buffer allows tracking of pipetted samples in the qPCR. When added to the blue QuantiNova Probe PCR Master Mix, the color changes from blue to green, indicating the successful inclusion of template. The use of this buffer is optional. It is provided as 100x concentrate and should be diluted (using water or Tris buffer) to obtain a final concentration of 1x within samples. To generate a template dilution series (e.g., for absolute quantification or determination of PCR efficiency), dilute the 100x concentrate (using water or Tris buffer) to obtain a final concentration of 1x QuantiNova Yellow Template Dilution Buffer. The buffer does not affect the sample stability and qPCR.
- For the highest efficiency in real-time PCR using TaqMan probes, amplicons should ideally be 60–150 bp in length.
- Before performing duplex analyses, choose suitable combinations of reporter dyes and quenchers that are compatible with duplex analysis using the detection optics of your real-time cycler. We strongly recommend using nonfluorescent quenchers.
- Always start with the cycling conditions and primer concentrations specified in this protocol.
- The PCR must start with an initial incubation step of 2 min at 95°C to activate the QuantiNova DNA Polymerase.
- For ease of use, we recommend preparing a 20x primer–probe mix containing target-specific primers and probe for each of your targets. A 20x primer–probe mix consists of 8 µM forward primer, 8 µM reverse primer and 4 µM probe in TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primer and probe solutions.

1. Thaw QuantiNova Probe PCR Master Mix, QuantiNova Yellow Template Dilution Buffer, template gDNA or cDNA, primers, probes, QN ROX Reference Dye (if required) and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 1. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 1. Reaction setup

	Volume/reaction		
Component	**96-well block, Rotor-Gene**	**384-well block**	**Final concentration**
Reaction mix			
2x Probe PCR Master Mix	10 µl	5 µl	1x
QN ROX Dye Solution (AB instruments only)	1 µl/0.1 µl*	0.5 µl/0.05 µl*	1x
20x primer–probe mix 1	1 µl	0.5 µl	0.4 µM forward primer 1 0.4 µM reverse primer 1 0.2 µM TaqMan probe 1
20x primer–probe mix 2	1 µl	0.5 µl	0.4 µM forward primer 2 0.4 µM reverse primer 2 0.2 µM TaqMan probe 2
RNase-free water	Variable	Variable	–
Template gDNA or cDNA (added at step 4)	Variable	Variable	≤100 ng/reaction
Total reaction volume	**20 µl**	**10 µl**	**–**

* Use a 1:20 dilution for high ROX cyclers (i.e., Applied Biosystems 7000, 7300, 7900 and StepOne Real-Time PCR Systems, and a 1:200 dilution for low-ROX dye cyclers (i.e., Applied Biosystems 7500 and ViiA7 Real-Time PCR Systems).

3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes, PCR capillaries or the wells of a PCR plate.
4. Add template gDNA or cDNA (≤100 ng/reaction) to the individual PCR tubes, capillaries or wells containing the reaction mix.

   Note: For two-step RT-PCR, the volume of the cDNA added (from the undiluted reverse-transcription reaction) should not exceed 15% of the final PCR volume.
5. Program the real-time cycler according to Table 2.

   Note: Data acquisition should be performed during the combined annealing/extension step.
6. 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	5 s	95°C	Maximal/fast mode
Combined annealing/extension		60°C	Maximal/fast mode
Singleplex	5 s*		
Duplex	30 s		
Number of cycles	40†		

* If your cycler does not accept this short time for data acquisition, use the shortest acceptable time.

† The number of cycles depends on the amount of template DNA.

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