Quick-Start Protocol May 2016

QuantiNova® Multiplex PCR Kit

The QuantiNova Multiplex PCR Kit (cat. nos. 208452, 208454, 208456) should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer and protected from light. QuantiNova Multiplex PCR Master Mix can also be stored protected from light at 2–8°C for up to 12 months, depending on the expiration date.

Further information

- *QuantiNova Multiplex PCR Kit Handbook*: **www.qiagen.com/HB-2105**
- 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 in a multiplex format (up to 5-plex) using TaqMan® probes with any real-time cycler and conditions 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, 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.

- QuantiNova 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. ROX dye should be diluted 1:20 for a 1x reaction when using

an instrument requiring a high ROX dye concentration. For instruments requiring a low ROX dye concentration, dilute the dye 1:200.

- The reference dye in QuantiNova Yellow Template Dilution Buffer allows tracking of pipetted samples in the qPCR. When template is added to the blue QuantiNova Multiplex PCR Master Mix, the color changes from blue to green. The use of this buffer is optional. It is provided as a 100x concentrate and should be diluted (using water) to obtain a 1x final concentration within the sample. To generate a template dilution series (e.g., for absolute quantification or determination of PCR efficiency), dilute the 100x concentrate (using template and water) to obtain a final concentration of 1x QuantiNova Yellow Template Dilution Buffer. The buffer does not affect 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 multiplex analyses, choose suitable combinations of reporter dyes and quenchers that are compatible with multiplex analysis using the detection optics of your real-time cycler. We strongly recommend using non-fluorescent 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 probes for each target. A 20x primer–probe mix consists of 8 µM forward primer, 8 µM reverse primer and 5 µM probe in TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primer and probe solutions.
- For information on suitable combinations of dyes for multiplex PCR using various cyclers, please refer to the *QuantiNova Multiplex PCR Kit Handbook*.
- For multiplex analyses, we strongly recommend using dual-labeled probes with nonfluorescent quenchers.

1. Thaw 4x QuantiNova Multiplex 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 mix setup

Component	96-well block, Rotor-Gene	384-well block	Final concentration
4x Multiplex PCR Master Mix	5 µl	2.5 µl	1x
QN ROX Reference Dye (AB instruments only)	1 µl/0.1 µl*	0.5 µl/0.05 µl*	1x
20x primer–probe mix† (for each of up to 5 targets)	1 µl	0.5 µl	0.4 µM forward primer 0.4 µM reverse primer 0.25 µM probe
RNase-free water	Variable	Variable	–
Template gDNA or cDNA (added at step 4)	Variable	Variable	≤800 ng/reaction
Total reaction volume	**20 µl**	**10 µl**	–

*Results in a 1:20 dilution for high ROX dye cyclers (i.e., ABI PRISM 7000, Applied Biosystems 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 and QuantStudio Real-Time PCR Systems) in the final 1x reaction.

† A 20x primer–probe mix consists of 8 µM forward primer, 8 µM reverse primer and 5 µM probe in TE buffer for each target. Primers and probes can either be pre-mixed and added simultaneously or primer-probe mixes for each target can be added separately. If concentration of primer-probe mix(es) differ, the respective added volume needs to be adjusted to achieve a final concentration of 0.4 µM for each primer and 0.25 µM for each probe.

3. Mix the reaction thoroughly and dispense appropriate volumes into PCR tubes or wells of a PCR plate.
4. Add template gDNA or cDNA (≤800 ng/reaction) to the individual PCR tubes or wells containing the reaction mix.

5. Program the real-time cycler according to Table 2.

   Note: Data acquisition should be performed during the combined annealing/extension step.

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	30 s	60°C	Maximal/fast mode
Number of cycles	**40***		

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

6. Place the PCR tubes or plates in the real-time cycler and start the cycling program.
7. Perform data analysis.

   Note: Before performing data analysis, select the analysis settings for each probe (i.e., baseline settings and threshold values). Optimal analysis settings are a prerequisite for accurate quantification data.

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