Quick-Start Protocol March 2016

QuantiFast® Probe PCR Kit

The QuantiFast Probe PCR Kit (cat. nos. 204254, 204256 and 204257) should be stored immediately upon receipt at –30°C to –15°C and protected from light. 2x QuantiFast Probe PCR Master Mix can also be stored protected from light at 2–8°C for up to 2 months, depending on the expiration date.

Further information

- *QuantiFast Probe PCR Handbook*: **www.qiagen.com/HB-0458**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for quantification of gDNA and cDNA targets using dual-labeled probes (e.g., TaqMan® probes) on any cycler from Applied Biosystems except the Applied Biosystems® 7500 and ViiA™ 7. PCR is carried out in the presence of ROX passive reference dye, which is included in 2x QuantiFast Probe PCR Master Mix.
- For the highest efficiency in real-time PCR using sequence-specific probes, targets should ideally be 70–200 bp in length.
- Always start with the cycling conditions specified in this protocol, even if using previously established primer–probe systems.
- The PCR must start with an initial incubation step of 3 min at 95°C to activate HotStarTaq® *Plus* DNA Polymerase.

1. Thaw 2x QuantiFast Probe PCR Master Mix, template gDNA or cDNA, primer and probe solutions 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.

   Note: We strongly recommend starting with the Mg^{2+} concentration as provided in 2x QuantiFast Probe PCR Master Mix.
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 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 10% of the final PCR volume.

Table 1. Reaction setup

	Volume/reaction		
Component	**96-well block**	**384-well block**	**Final concentration**
Reaction mix 2x QuantiFast Probe PCR Master Mix	12.5 µl	5 µl	1x
Primer A	Variable	Variable	0.4 µM
Primer B	Variable	Variable	0.4 µM
Probe	Variable	Variable	0.2 µM
RNase-free water	Variable	Variable	–
Template gDNA or cDNA (added at step 4)	Variable	Variable	≤200 ng/reaction
Total reaction volume	25 µl	10 µl	

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, capillaries 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	3 min	95°C	Maximal/fast mode
2-step cycling:			
Denaturation	3 s*	95°C	Maximal/fast mode
Combined annealing/extension	30 s*	60°C	Maximal/fast mode
Number of cycles	35–40†		

* If your cycler does not accept this short time, choose the shortest time possible (e.g., 5 s denaturation for the ABI PRISM® 7700 or 31 s annealing/extension for the ABI PRISM 7000 or Applied Biosystems 7300).

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

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