Quick-Start Protocol March 2016

QuantiFast® Probe PCR +ROX Vial Kit

The QuantiFast Probe PCR +ROX Vial Kit (cat. nos. 204354 and 204356) should be stored immediately upon receipt at –30°C to –15°C and protected from light. 2x QuantiFast Probe PCR Master Mix (w/o ROX) 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) with the Applied Biosystems® 7500, Applied Biosystems ViiA™ 7 and real-time cyclers from Bio-Rad, Cepheid, Eppendorf, Roche and Agilent/Stratagene and using FRET probes with the LightCycler® 1.x and LightCycler 2.0.
- Use of the supplied ROX passive reference dye solution is necessary for the Applied Biosystems 7500. ROX dye solution is not required for all other cyclers.
- 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 (w/o ROX), template gDNA or cDNA, primer and probe solutions, ROX dye solution (if needed) and RNase-free water. Mix the individual solutions.

   Optional: If you always run reactions with ROX dye, you can premix 50x ROX Dye Solution with 2x QuantiFast Probe PCR Master Mix (w/o ROX) according to Table 1. Master Mix containing ROX dye can be stored at 2–8°C for up to 2 months, or at –15°C to –30°C until the indicated expiration date.

Table 1. Option for users of the Applied Biosystems 7500, Applied Biosystems ViiA 7, Mx3000P™, Mx3005P™ and Mx4000® — Addition of ROX dye to Master Mix for long-term storage

Kit	Volume of 50x ROX Dye Solution	Volume of 2x QuantiFast Probe PCR Master Mix (w/o ROX)
QuantiFast Probe PCR +ROX Vial Kit (400)	68 µl	1.7 ml
QuantiFast Probe PCR +ROX Vial Kit (2000)	1 ml	25 ml

2. Prepare a reaction mix according to Table 2. 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 (w/o ROX).

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 2. Reaction setup

Component	Volume/reaction			Final conc.
	96-well block	Fast or capillary*	384-well block	
Reaction mix				
2x QuantiFast Probe PCR Master Mix (w/o ROX)	12.5 µl	10 µl	5 µl	1x
Primer A	Variable	Variable	Variable	0.4 µM†
Primer B	Variable	Variable	Variable	0.4 µM†
Probe	Variable	Variable	Variable	0.2 µM‡
50x ROX Dye Solution§¶	0.5 µl	0.4 µl	–**	1x
RNase-free water	Variable	Variable	Variable	–
Template gDNA or cDNA (added at step 4)	Variable	Variable	Variable	≤200 ng/ reaction
Total reaction volume	25 µl	20 µl	10 µl	

* Applied Biosystems 7500 Fast System or capillary cycler.

† Reactions using dual-labeled probes. For reactions using FRET probes, use a final concentration of 0.6 µM for each primer.

‡ Reactions using dual-labeled probes. For reactions using FRET probes, use a final concentration of 0.2 µM for each probe.

§ Required for Applied Biosystems 7500 and ViiA 7 and optional for Mx3000P, Mx3005P and Mx4000.

¶ For cyclers which do not require ROX dye, add RNase-free water instead.

** A total reaction volume of 10 µl is strongly recommended for the LightCycler® 480, which does not require ROX dye.

5. Program the real-time cycler according to Table 3 or, if using FRET probes with the LightCycler 1.x or LightCycler 2.0, Table 4.

   Note: Data acquisition should be performed during the combined annealing/extension (2-step cycling) or annealing (3-step cycling) step.

6. Place the PCR tubes, capillaries or plates in the real-time cycler, and start the cycling program.

Table 3. Cycling conditions for dual-labeled probes

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*		

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

Table 4. Cycling conditions for FRET probes on the LightCycler 1.x and 2.0

Step	Time	Temperature	Ramp rate
PCR initial heat activation	3 min	95°C	Maximal/fast mode
3-step cycling:			
Denaturation	10 s	95°C	Maximal/fast mode
Annealing	15 s	50–60°C	Maximal/fast mode
Extension	15 s	72°C	Maximal/fast mode
Number of cycles	35–40†		

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

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