Quick-Start Protocol March 2016

QuantiFast® Probe RT-PCR Plus Kit

The QuantiFast Probe RT-PCR Plus Kit (cat. nos. 204482 and 204484) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. When the kit is stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the quality-control label inside the kit box or on the kit envelope). The 2x QuantiFast Mix 2 (Probe) can also be stored protected from light at 2–8°C for up to 1 month without showing any reduction in performance. If required, ROX™ Dye Solution or High-ROX Dye Solution can be added to 2x QuantiFast Mix 2 (Probe) (w/o ROX) for long-term storage.

Further information

- *QuantiFast Probe RT-PCR Plus Handbook*: **www.qiagen.com/HB-0430**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- High-ROX Dye Solution is for use with real-time cyclers from Applied Biosystems except Applied Biosystems® 7500 Real-Time PCR Systems and the ABI ViiA7. For Applied Biosystems 7500 Real-Time PCR Systems, the ABI ViiA7 and cyclers from Agilent, ROX dye is required at a lower concentration. For more information, refer to "Passive reference dye", page 12, of the *QuantiFast Probe RT-PCR Plus Handbook*.
- If using an already established duplex real-time RT-PCR assay, use the previously established primer and probe concentrations in combination with the cycling conditions specified in this protocol. It is not necessary to determine the primer limiting concentrations again.

- For optimal results in duplex RT-PCR, follow the recommended combinations of dyes shown in Tables 3–14 (pages 19–30) of the *QuantiFast Probe RT-PCR Plus Handbook* and choose suitable combinations of reporter dyes and quenchers that are compatible with duplex analysis using the detection optics of your real-time cycler. Order the probes from an established oligonucleotide manufacturer.
- We strongly recommend testing the performance of primer-probe sets in individual assays before combining them in a duplex assay.
- If using QuantiFast Probe Assays, reconstitute with TE buffer according to the instructions provided in the product sheet. After reconstitution, QuantiFast Probe Assays (20x) can be used in the following protocol.

1. Thaw 2x QuantiFast Mix 1, 2x QuantiFast Mix 2 (Probe), template RNA, primer and probe solutions, RNase-free water and High-ROX Dye Solution or ROX Dye Solution. Mix the individual solutions. QuantiFast RT Mix should be taken from –30 to –15°C immediately before use, and returned to storage at –30 to –15°C immediately after use.
2. Prepare a reaction mix for removal of genomic DNA according to Table 1.

Table 1. Reaction setup for genomic DNA removal

Component	Volume/reaction	Final concentration
2x QuantiFast Mix 1	6.25 µl	1x
Template RNA (added at step 4)	Variable	≤100 ng/reaction
RNase-free water	Variable	
Total reaction volume	**13 µl***	

* Related to the final volume for the QuantiFast real-time PCR reaction of 25 µl.

3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes, PCR capillaries or the wells of a PCR plate.

   Note: Do not keep the PCR vessels or plates on ice.
4. Add template RNA (≤100 ng) to the individual PCR tubes, PCR capillaries or wells and incubate for 5 min at room temperature (15–25°C).

   Note: The incubation step can be prolonged up to 15 min.
5. Prepare a QuantiFast Reaction mix according to Table 2.

6. Mix the QuantiFast reaction mix thoroughly and dispense appropriate volumes into PCR vessels or plates containing the genomic DNA removal reaction.

Note: If the transfer to the real-time cycler needs more than 5 min, store the plates on ice.

Table 2. Setup of QuantiFast reaction mix

Component	Most Applied Biosystems cyclers* Volume/reaction‡	Rotor-Gene cyclers, Applied Biosystems 7500, ViiA7 and other cyclers† Volume/reaction‡	Final concentration§
2x QuantiFast Mix 2 (Probe)	6.25 µl	6.25 µl	1x
20x primer-probe mix 1¶ **or** 20x QuantiFast Probe Assay (FAM™)	1.25 µl	1.25 µl	0.4 µM forward primer 1** 0.4 µM reverse primer 1** 0.2 µM probe 1††
20x primer-probe mix 2¶ **or** 20x QuantiFast Probe Assay (MAX)	1.25 µl	1.25 µl	0.4 µM forward primer 2** 0.4 µM reverse primer 2** 0.2 µM probe 2††
High-ROX Dye Solution‡‡	0.5 µl	–	
ROX Dye Solution‡‡	–	0.5 µl	
QuantiFast RT Mix	0.25 µl	0.25 µl	
RNase-free water	2.5 µl	2.5 µl	
Total reaction volume	**12 µl‡**	**12 µl‡**	

* All Applied Biosystems cyclers except Applied Biosystems 7500 Real-Time Systems and the ABI ViiA7.

† All other cyclers, including Rotor-Gene cyclers, Applied Biosystems 7500 Real-Time PCR Systems, the ABI ViiA7 and real-time cyclers from Bio-Rad, Cepheid, Eppendorf, Roche and Agilent.

‡ If your real-time cycler requires a final reaction volume other than 25 µl, adjust the amount of master mix and all other reaction components accordingly. If using 384-well plates on the Applied Biosystems 7900HT, use a reaction volume of 10 µl.

§ For primer-probe assays other than QuantiFast Probe Assays.

¶ A 20x primer–probe mix for duplex RT-PCR consists of 8 µM forward primer, 8 µM reverse primer and 4 µM probe in TE buffer.

** A final primer concentration of 0.4 µM is optimal. Before adapting primer concentration, check the concentration of your primer solutions.

†† A final probe concentration of 0.2 µM gives satisfactory results in most cases. Depending on the synthesis quality and purification method used, the optimal concentration may be between 0.1 µM and 0.4 µM.

‡‡ For cyclers that do not require ROX dye, add RNase-free water instead.

7. Program the real-time cycler according to Table 3.

   Note: Check the real-time cycler's user manual for correct instrument setup for multiplex analysis. Be sure to activate the detector for each reporter dye used. Depending on your instrument, it may be also necessary to perform a calibration procedure for each of the reporter dyes before they are used for the first time.

Table 3. Cycling conditions

Step	Time	Temperature	Additional comments
Reverse transcription	20 min	50°C	RNA is reverse transcribed into cDNA
PCR initial activation step	5 min	95°C	HotStarTaq® *Plus* DNA Polymerase is activated by this step
2-step cycling			**Important**: Optimal performance is only assured using these cycling conditions
Denaturation	15 s	95°C	
Annealing/extension	30 s	60°C	Combined annealing/extension step with fluorescence data collection
Number of cycles	40–45		The number of cycles depends on the amount of template RNA and the expression level of the target gene.

8. Perform data analysis. 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.

Scan QR code for handbook.

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