Quick-Start Protocol March 2016

QuantiFast® Multiplex RT-PCR Kit

The QuantiFast Multiplex RT-PCR Kit (cat. nos. 204852 and 204854) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. 2x QuantiFast Multiplex RT-PCR Master Mix can also be stored protected from light at 2–8°C for up to 1 month, depending on the expiration date.

Further information

- *QuantiFast Multiplex RT-PCR Handbook*: **www.qiagen.com/HB-0178**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for quantification of RNA targets for gene expression analysis in a multiplex format, using TaqMan®/hydrolysis probes and any real-time cycler from Applied Biosystems except Applied Biosystems® 7500 Real-Time PCR Systems. Using this protocol, duplex, triplex or 4-plex RT-PCR is carried out in the presence of ROX passive reference dye, which is included in 2x QuantiFast Multiplex RT-PCR Master Mix.
- We recommend preparing a 20x primer–probe mix for each target containing target-specific primers and probe (see Table 1).
- For information on suitable combinations of reporter dyes for multiplex RT-PCR on various cyclers, please refer to the *QuantiFast Multiplex RT-PCR Handbook*.
- For multiplex analyses, we strongly recommend using dual-labeled probes with nonfluorescent quenchers.
- Set up all reactions on ice.

- After reverse transcription, the PCR must start with an initial incubation step of 5 min at 95°C to activate HotStarTaq® *Plus* DNA Polymerase.

1. Thaw 2x QuantiFast Multiplex RT-PCR Master Mix, template RNA, primer and probe solutions and RNase-free water. Mix the individual solutions, and place them on ice. QuantiFast RT Mix should be taken from –30 to –15°C immediately before use, always kept on ice, and returned to storage at –30 to –15°C immediately after use.
2. Prepare a reaction mix according to Table 1.

   Note: We strongly recommend starting with the optimized Mg^{2+} concentration provided by 2x QuantiFast Multiplex RT-PCR Master Mix.
3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.
4. Add template RNA (≤100 ng) to the individual PCR tubes or wells.

Table 1. Reaction setup for multiplex RT-PCR

Component	Volume/reaction
Reaction mix	
2x QuantiFast Multiplex RT-PCR Master Mix	12.5 µl
20x primer–probe mix 1*	1.25 µl
20x primer–probe mix 2*	1.25 µl
Only for triplex and 4-plex RT-PCR: 20x primer–probe mix 3*	1.25 µl
Only for 4-plex RT-PCR: 20x primer–probe mix 4*	1.25 µl
QuantiFast RT Mix	0.25 µl
RNase-free water	Variable
Template RNA (added at step 4)	Variable (≤100 ng/reaction)
Total reaction volume	25 µl†

* **IMPORTANT**: For duplex, triplex and 4-plex RT-PCR, a 20x primer-probe mix consists of 8 µM forward primer, 8 µM reverse primer and 4 µM probe in TE buffer, resulting in a final concentration of 0.4 µM forward primer and reverse primer and 0.2 µM probe.

† If your real-time cycler requires a final reaction volume other than 25 µl, adjust the amount of 2x QuantiFast Multiplex RT-PCR 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.

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

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

6. Place the PCR tubes or plate 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.

Table 2. Cycling conditions

Step	Time	Temperature
Reverse transcription	20 min	50°C
PCR initial heat activation	5 min	95°C
2-step cycling:		
Denaturation	15 s	95°C
Annealing/extension	30 s	60°C
Number of cycles	40–45*	

* The number of cycles depends on the amount of template RNA and the expression level of the target gene.

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