Quick-Start Protocol March 2016

QuantiTect® Multiplex PCR Kit

The QuantiTect Multiplex PCR Kit (cat. nos. 204541, 204543 and 204545) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. 2x QuantiTect Multiplex PCR Master Mix can also be stored protected from light at 2–8°C for up to 6 months, depending on the expiration date.

Further information

- *QuantiTect Multiplex PCR Handbook*: **www.qiagen.com/HB-0881**
- 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 in a multiplex format, using TaqMan®/hydrolysis probes with real-time cyclers from Applied Biosystems. Using this protocol, duplex, triplex or 4-plex PCR is carried out in the presence of ROX passive reference dye, which is included in 2x QuantiTect Multiplex PCR Master Mix.
- 2x QuantiTect Multiplex PCR Master Mix contains dUTP, which allows the use of a uracil-N-glycosylase (UNG) pretreatment of the reaction if contamination with carried-over PCR products is suspected.
- We recommend preparing a 20x primer–probe mix for each target containing target-specific primers and probe (see Tables 1 and 2).
- For information on suitable combinations of reporter dyes for multiplex PCR on various cyclers, please refer to the *QuantiTect Multiplex PCR Handbook*.
- For multiplex analyses, we strongly recommend using dual-labeled probes with nonfluorescent quenchers.

- The PCR must start with an initial incubation step of 15 min at 95°C to activate HotStarTaq® DNA Polymerase.

1. Thaw 2x QuantiTect Multiplex PCR Master Mix, template gDNA or cDNA, primer and probe solutions and RNase-free water. Mix the individual solutions, and place them on ice.
2. Prepare a reaction mix according to Table 1 for duplex PCR and Table 2 for triplex and 4-plex PCR.

   Note: We strongly recommend starting with the optimized Mg^{2+} concentration provided by 2x QuantiTect Multiplex PCR Master Mix. For a very limited number of targets, reactions may be improved by increasing the final Mg^{2+} concentration by 0.5–1 mM.
3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.

Table 1. Reaction setup for duplex PCR on Applied Biosystems® cyclers

Component	Volume/reaction
Reaction mix	
2x QuantiTect Multiplex PCR Master Mix	25 µl
20x primer–probe mix 1*	2.5 µl
20x primer–probe mix 2*	2.5 µl
RNase-free water	Variable
Optional: Uracil-N-glycosylase†	Variable (0.5 units/reaction)
Template gDNA or cDNA (added at step 4)	Variable (≤500 ng/reaction)
Total reaction volume	50 µl‡

* **IMPORTANT**: For duplex PCR on Applied Biosystems cyclers, 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 and reverse primer and 0.2 µM probe.

† The activity of uracil-N-glycosylase may differ between suppliers. Depending on your supplier, the optimal concentration may be 0.25–1 units per 50 µl reaction.

‡ If your real-time cycler requires a final reaction volume other than 50 µl, adjust the amount of 2x QuantiTect Multiplex PCR Master Mix and all other reaction components accordingly. If using 384-well plates on the ABI PRISM® 7900, use a reaction volume of 20 µl.

Table 2. Reaction setup for triplex and 4-plex PCR on Applied Biosystems cyclers

Component	Volume/reaction
Reaction mix	
2x QuantiTect Multiplex PCR Master Mix	25 µl
20x primer–probe mix 1*	2.5 µl
20x primer–probe mix 2*	2.5 µl
20x primer–probe mix 3*	2.5 µl
Only for 4-plex PCR:	
20x primer–probe mix 4*	2.5 µl
RNase-free water	Variable
Optional: Uracil-N-glycosylase†	Variable (0.5 units/reaction)
Template gDNA or cDNA (added at step 4)	Variable (≤250 ng/reaction)
Total reaction volume	50 µl‡

* **IMPORTANT**: For triplex and 4-plex PCR on Applied Biosystems cyclers, a 20x primer–probe mix consists of 4 µM forward primer, 4 µM reverse primer and 4 µM probe in TE buffer, resulting in a final concentration of 0.2 µM forward primer, reverse primer and probe.

† The activity of uracil-N-glycosylase may differ between suppliers. Depending on your supplier, the optimal concentration may be 0.25–1 units per 50 µl reaction.

‡ If your real-time cycler requires a final reaction volume other than 50 µl, adjust the amount of 2x QuantiTect Multiplex PCR Master Mix and all other reaction components accordingly. If using 384-well plates on the ABI PRISM 7900, use a reaction volume of 20 µl.

4. Add template gDNA or cDNA to the individual PCR tubes or wells.

   Note: For two-step RT-PCR, the volume of cDNA (from the undiluted reverse-transcription reaction) added as template should not exceed 10% of the final PCR volume.

5. 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 (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.

Note: If using the Applied Biosystems 7500, it is necessary to adjust the preset threshold value to a lower value. Use a value of 0.01as a starting point.

Table 3. Cycling conditions

Step	Time	Temperature
Optional: UNG pretreatment (if UNG included in reaction mix)	2 min	50°C
PCR initial heat activation	15 min	95°C
2-step cycling: Denaturation	60 s	94°C
Annealing/extension Duplex PCR	60 s	60°C
Triplex and 4-plex PCR	90 s	60°C
Number of cycles	40–50*	

* The number of cycles depends on the amount of template gDNA or cDNA and, for cDNA targets, the expression level of the target gene.

Scan QR code for handbook.

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

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