March 2020

Quick-Start Protocol

QuantiTect® Probe PCR Kit

The QuantiTect Probe PCR Kit (cat. nos. 204343 and 204345) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The 2x QuantiTect Probe 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 Probe PCR Handbook*: **www.qiagen.com/HB-0233**
- 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 (including TaqMan® probes, FRET probes, and Molecular Beacons) with most real-time cyclers. PCR is carried out in the presence of ROX passive reference dye, which is included in the 2x QuantiTect Probe PCR Master Mix and is necessary for all real-time cyclers from Applied Biosystems®. The presence of ROX dye does not interfere with real-time PCR on any other instrument.
- For the highest efficiency in real-time PCR using sequence-specific probes, targets should ideally be 100–150 bp in length.
- The 2x QuantiTect Probe 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.
- 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 15 min at 95°C to activate HotStarTaq® DNA Polymerase.
- Always readjust the threshold value for analysis of every run.

Procedure

1. Thaw 2x QuantiTect Probe PCR Master Mix (if stored at –30 to –15°C), template gDNA or cDNA, primer and probe solutions, and RNase-free water. Mix 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 an initial Mg^{2+} concentration of 4 mM as provided by 2x QuantiTect Probe PCR Master Mix. For a very limited number of targets, reactions may be improved by using Mg^{2+} concentrations of up to 6 mM.
3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes, PCR capillaries or the wells of a PCR plate.

Table 1. Reaction setup

	LightCycler® 1.x and 2.0		Other cyclers*	
Component	**Volume**	**Final conc.**	**Volume**	**Final conc.**
Reaction mix				
2x QuantiTect Probe PCR Master Mix†	10 µl‡	1x	25 µl‡	1x
Primer A	Variable	0.5 µM	Variable	0.4 µM
Primer B	Variable	0.5 µM	Variable	0.4 µM
Probe	Variable	0.1–0.2 µM	Variable	0.1–0.2 µM
RNase-free water	Variable	–	Variable	–
Optional: Uracil-N-glycosylase, heat-labile	Variable	0.5 units/reaction	Variable	0.5 units/reaction
Template gDNA or cDNA (added at step 4)	Variable	≤1 µg/reaction	Variable	≤500 ng/reaction
Total reaction volume	**20 µl**		**50 µl**	

* Includes Rotor-Gene® cyclers and instruments from Applied Biosystems, Bio-Rad, Cepheid, Eppendorf, Roche, and Agilent/Stratagene.

† Provides a final concentration of 4 mM $MgCl_2$.

‡ If using a total reaction volume other than indicated, adjust the volume of 2x QuantiTect Probe PCR Master Mix accordingly.

4. Add template gDNA or cDNA to the individual PCR tubes, capillaries, or wells containing the reaction mix.

   Note: For 2-step RT-PCR, the volume of the cDNA added (from the undiluted reverse-transcription reaction) should not exceed 10% of the final PCR volume.

5. Program the real-time cycler according to Table 2 or, if using FRET probes on the LightCycler 1.x or LightCycler 2.0, Table 3.

   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 or plates in the real-time cycler, and start the cycling program.
   Note: If using the Applied Biosystems 7500, it is necessary to adjust the preset threshold value to a lower value. Use a value of 0.01 as a starting point.
   Note: If using the LightCycler 1.x or 2.0, we recommend using the "second derivative maximum" method for data analysis. Always readjust the noise band for analysis of every run if using the "fit-point" method for data analysis.

Table 2. Cycling conditions for dual-labeled probes

	LightCycler 1.x and 2.0		Other cyclers*	
Step	**Time***	**Temperature**	**Time**	**Temperature**
Optional: UNG pretreatment (if UNG is included in reaction mix)	2 min	50°C	2 min	50°C
PCR initial heat activation	15 min	95°C	15 min	95°C
2-step cycling:				
Denaturation	0 s	95°C	15 s†	94°C
Combined annealing/extension	60 s	60°C	60 s	60°C
Number of cycles	35–55‡		35–45‡	

* Ramp rate: 20°C/s.

† SmartCycler® users can reduce denaturation time to 1 s to take advantage of cycling capacities.

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

Table 3. Cycling conditions for FRET probes on the LightCycler 1.x and 2.0

Step	Time	Temperature	Ramp rate
Optional: UNG pretreatment (if UNG is included in reaction mix)	2 min	50°C	20°C/s
PCR initial heat activation	15 min	95°C	20°C/s
3-step cycling:			
Denaturation	0 s	95°C	20°C/s
Annealing	30 s	50–60°C	20°C/s
Extension	30 s	72°C	2°C/s
Number of cycles	35–55*		

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

Document Revision History

Date	Changes
03/2020	Removed QuantiTect RT Mix from Table 1. Removed cat. no. 204341 in first paragraph, because product has been discontinued. Revised final conc. of optional UNG in Table 1 (from 0.2 units/reaction, it is now 0.5 units/reaction) to maintain consistency with handbook.

Scan QR code for handbook.

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