Quick-Start Protocol March 2016

QuantiTect® Probe RT-PCR Kit

The QuantiTect Probe RT-PCR Kit (cat. nos. 204443 and 204445) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. 2x QuantiTect Probe RT-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 RT-PCR Handbook*: **www.qiagen.com/HB-0234**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for quantification of RNA targets in gene expression analysis, using dual-labeled probes (including TaqMan® probes, FRET probes and Molecular Beacons) and most real-time cyclers. PCR is carried out in the presence of ROX passive reference dye, which is included in 2x QuantiTect Probe RT-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.
- Set up all reactions on ice to avoid premature cDNA synthesis.
- 2x QuantiTect Probe RT-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. Only heat-labile UNG should be used.
- Always start with the cycling conditions specified in this protocol, even if using previously established primer–probe systems.
- After reverse transcription, the PCR step of the RT-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.

1. Thaw 2x QuantiTect Probe RT-PCR Master Mix (if stored at –20°C), template RNA, primer and probe solutions and RNase-free water. Mix the individual solutions and place them on ice. QuantiTect 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 an initial Mg^{2+} concentration of 4 mM as provided by 2x QuantiTect Probe RT-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 RT-PCR Master Mix†	10 µl‡	1x	25 µl‡	1x
Primer A	Variable	1 µM	Variable	0.4 µM
Primer B	Variable	1 µM	Variable	0.4 µM
Probe	Variable	0.2 µM	Variable	0.1–0.2 µM§
QuantiTect RT Mix	0.2 µl‡		0.5 µl‡	
RNase-free water	Variable	–	Variable	–
Optional: Uracil-N-glycosylase, heat-labile	Variable	2 units/reaction	Variable	2 units/reaction
Template RNA (added at step 4)	Variable	1 pg–1 µg/ reaction	Variable	1 pg–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 RT-PCR Master Mix and QuantiTect RT Mix accordingly.

§ SmartCycler® users should use a final probe concentration of 0.2 µM.

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

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.

Table 2. Cycling conditions for dual-labeled probes

	LightCycler® 1.x and 2.0		**Other cyclers**	
Step	**Time***	**Temperature**	**Time**	**Temperature**
Reverse transcription	20 min	50°C	30 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‡		40–45‡	

* 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 RNA and the expression level of the target gene.

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

Step	**Time**	**Temperature**	**Ramp rate**
Reverse transcription	20 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 RNA and the expression level of the target gene.

6. Place the PCR tubes, capillaries 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 and always readjust the noise band for analysis of every run if using the "fit-point" method for data analysis.

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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