Quick-Start Protocol March 2016

QuantiTect® SYBR® Green PCR Kit

The QuantiTect SYBR Green PCR Kit (cat. nos. 204141, 204143 and 204145) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. 2x QuantiTect SYBR Green 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 SYBR Green PCR Handbook*: **www.qiagen.com/HB-0231**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for quantification of gDNA or cDNA targets using SYBR Green I with any real-time cycler. PCR is carried out in the presence of ROX passive reference dye, which is included in 2x QuantiTect SYBR Green PCR Master Mix and is necessary for real-time cyclers from Applied Biosystems. The presence of ROX dye does not interfere with real-time PCR using other instruments.
- For the highest efficiency in real-time PCR using SYBR Green I, targets should ideally be 100–150 bp in length.
- 2x QuantiTect SYBR Green 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.
- 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.
- If using QuantiTect Primer Assays, please follow the protocols in the *QuantiTect Primer Assay Handbook*, which can be downloaded at **www.qiagen.com/HB-0169**.

- If using the iCycler iQ®, iQ5 or MyiQ, well factors must be collected at the beginning of each experiment. Well factors are used to compensate for any system or pipetting nonuniformity. For details, refer to the user manual supplied with the instrument or the *QuantiTect SYBR Green PCR Handbook*.

1. Thaw 2x QuantiTect SYBR Green PCR Master Mix (if stored at –30 to –15°C), template gDNA or cDNA, primers and RNase-free water. Mix the 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 2.5 mM as provided by 2x QuantiTect SYBR Green PCR Master Mix. For a very limited number of targets, reactions may be improved by using Mg^{2+} concentrations of up to 5 mM.
3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR vessels or the wells of a PCR plate.

Table 1. Reaction setup

	Volume/reaction	
Component	**LightCycler® 1.x and 2.0**	**Other real-time cyclers***
Reaction mix		
2x QuantiTect SYBR Green PCR Master Mix†	10 µl	25 µl‡
Primer A	Variable (final concentration of 0.5 µM)	Variable (final concentration of 0.3 µM)
Primer B	Variable (final concentration of 0.5 µM)	Variable (final concentration of 0.3 µM)
RNase-free water	Variable	Variable
Optional: Uracil-N-glycosylase	Variable (0.2 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 2.5 mM $MgCl_2$.

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

4. Add template gDNA or cDNA to the individual PCR vessels or wells containing the reaction mix.

   Note: For two-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 LightCycler 1.x or LightCycler 2.0, Table 3.

   Note: Data acquisition should be performed during the extension step. After performing melting curve analysis (see step 7), an additional data acquisition step for further runs with the same target can be integrated (see steps 8 and 9).

6. Place the PCR tubes or plates in the real-time cycler, and start the cycling program.

   Note: If using the Applied Biosystems® 7500, we recommend adjusting the default **Manual Ct** threshold value of 0.2 to a lower value (e.g., 0.02) in order to analyze the data properly. For details, see Appendix B of the *QuantiTect SYBR Green PCR Handbook*.

Table 2. Cycling conditions (except LightCycler 1.x and LightCycler 2.0)

Step	Time	Temperature
Optional: UNG pretreatment	2 min	50°C
PCR initial heat activation	15 min	95°C
3 (4)-step cycling:		
Denaturation*	15 s	94°C
Annealing	30 s	50–60°C
Extension	30 s	72°C
Optional: Data acquisition	15 s	x°C†
Number of cycles	35–45‡	

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

† T_m dimer < x < T_m product; see step 8 for details.

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

Table 3. Cycling conditions for LightCycler 1.x and LightCycler 2.0

Step	Time	Temperature	Ramp rate
Optional: UNG pretreatment	2 min	50°C	20°C/s
PCR initial heat activation	15 min	95°C	20°C/s
3 (4)-step cycling:			
Denaturation	15 s	94°C	20°C/s
Annealing	20–30 s	50–60°C	20°C/s
Extension	10–30 s	72°C	2°C/s
Optional: Data acquisition	5 s	x°C*	20°C/s
Number of cycles	35–55†		

* T_m dimer < x < T_m product; see step 8 for details.

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

7. Perform a melting curve analysis of the PCR product(s).

   Note: We strongly recommend routinely performing this analysis, which is built into the software of real-time cyclers, to verify the specificity and identity of PCR products. For further information, please refer to the *QuantiTect SYBR Green PCR Handbook*.

8. **Optional**: Repeat the previous run, including an additional data acquisition step.

   Note: To suppress fluorescence readings caused by primer–dimers, an additional data acquisition step can be added to the 3-step cycling protocol (see Tables 2 and 3). The temperature should be above the T_m of primer–dimers but approximately 3°C below the T_m of the specific PCR product. For further details, please refer to the *QuantiTect SYBR Green PCR Handbook*.

9. **Optional**: Check the specificity of PCR product(s) by agarose gel electrophoresis.

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