Quick-Start Protocol March 2016

QuantiFast® SYBR® Green PCR Kit

The QuantiFast SYBR Green PCR Kit (cat. nos. 204054, 204056 and 204057) should be stored immediately upon receipt at –30°C to –15°C and protected from light. 2x QuantiFast SYBR Green 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 SYBR Green PCR Handbook*: **www.qiagen.com/HB-0460**
- 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 QuantiFast 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.
- The two-step PCR cycling protocol, which has a denaturation step at 95°C and a combined annealing/extension step at 60°C, will also work for primers with a T_m well below 60°C.
- For the highest efficiency in real-time PCR using SYBR Green I, targets should ideally be 60–200 bp in length.
- The PCR must start with an initial incubation step of 5 min at 95°C to activate HotStarTaq® *Plus* DNA Polymerase.

- 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 *QuantiFast SYBR Green PCR Handbook*.

1. Thaw 2x QuantiFast SYBR Green PCR Master Mix, 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 QuantiFast SYBR Green PCR Master Mix.
3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes, PCR capillaries or the wells of a PCR plate.
4. Add template gDNA or cDNA (≤100 ng/reaction) to the individual PCR tubes, capillaries 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.

Table 1. Reaction setup

	Volume/reaction			
Component	**96-well block**	**Capillary cycler**	**384-well block**	**Final conc.**
Reaction mix				
2x QuantiFast SYBR Green PCR Master Mix	12.5 µl	10 µl	5 µl	1x
Primer A*	Variable	Variable	Variable	1 µM
Primer B*	Variable	Variable	Variable	1 µM
RNase-free water	Variable	Variable	Variable	–
Template gDNA or cDNA (added at step 4)	Variable	Variable	Variable	≤100 ng/ reaction
Total reaction volume	25 µl	20 µl	10 µl	

* If using QuantiTect® Primer Assays, the final concentration in the reaction should be 1x.

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

   Note: Data acquisition should be performed during the combined annealing/extension step.

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

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 *QuantiFast SYBR Green PCR Handbook*.

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

Table 2. Cycling conditions

Step	Time	Temperature	Ramp rate
PCR initial heat activation	5 min	95°C	Maximal/fast mode
2-step cycling:			
Denaturation	10 s	95°C	Maximal/fast mode
Combined annealing/extension	30 s	60°C*	Maximal/fast mode
Number of cycles	35–40†		

* This temperature should also be used for QuantiTect Primer Assays and for all primer sets with a T_m well below 60°C.

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

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