Quick-Start Protocol May 2019

UCP SYBR® Green PCR Kit

The UCP SYBR® Green PCR Kit (cat. nos. 208012 and 208014) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. UCP SYBR® Green PCR Master Mix, UCP Yellow Template Dilution Buffer, and UCP ROX™ Reference Dye can also be stored at 2–8°C for up to 6 months, depending on the expiry date printed on the kit label. Since UCP (ultra-clean production) reagents are depleted from nucleic acids, appropriate measures should be taken to prevent any contamination during storage or use.

Further information

- *UCP SYBR® Green PCR Kit Handbook*: **www.qiagen.com/HB-2667**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The dye in UCP Yellow Template Dilution Buffer allows tracking of pipetted samples in the qPCR. When this buffer is added to the blue UCP SYBR® Green PCR Master Mix, the color of the Master Mix changes from blue to green, indicating the successful addition of the template. The use of this buffer is optional. It is provided as 100x concentrate and should be diluted (using UCP water) to obtain a final concentration of 1x within sample.* The buffer does not affect sample stability or qPCR.
- For the highest efficiency in real-time PCR using SYBR® Green I, amplicons should ideally be 60–200 bp in length. Longer systems such as Earth Microbiome primer sets are also possible with adapted protocol.

* Example: Add 0.5 µl Yellow Template Dilution Buffer to a 50 µl sample, which can be used as template in various PCR runs, regardless of the volume added to each reaction. Yellow Template Dilution Buffer can be prediluted using UCP water. In this example, add 5 µl of 1:10 prediluted Yellow Template Dilution Buffer.

- The 2-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 that is well below 60°C.
- Always start with the cycling conditions and primer concentrations specified in this protocol. We recommend using 1 µl of 20x primer mix per 20 µl reaction.
- The PCR must start with an initial incubation step of 2 min at 95°C to activate the hot-start DNA polymerase.
- This protocol is optimized for quantification of gDNA or cDNA targets using SYBR® Green I with any real-time cycler and condition for fluorescence normalization. The amount of required ROX dye varies, depending on the instrument used:
  No requirement for ROX dye: Rotor-Gene®, Bio-Rad® CFX, Roche® LightCycler® 480, and Agilent® Technologies Mx instruments
  Low concentration of ROX dye: Applied Biosystems® 7500, ViiA®7, and QuantStudio® Real-Time PCR Systems
  High concentration of ROX dye: ABI PRISM®7000, Applied Biosystems 7000, 7300, 7900, and StepOne® Real-Time PCR Systems
- Use appropriate techniques to avoid contamination of the ultra-clean reagents with nucleic acids, because this affects background signals in the nontemplate controls. Furthermore, primers should be designed to show no primer-dimer formation, because this might also result in signals in the nontemplate controls.

1. Thaw UCP SYBR® Green PCR Master Mix, UCP Yellow Template Dilution Buffer, template gDNA or cDNA, primers, probes, UCP ROX Reference Dye (if required), and UCP 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.
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 UCP SYBR® Green PCR Master Mix Kit

Component	Volume/reaction		Final concentration
	96-well	384-well block	
Reaction mix			
2x UCP SYBR® Green PCR Master Mix	10 µl	5 µl	1x
UCP ROX Reference Dye (Applied Biosystems cycler only)	1 µl/0.1 µl*	0.5 µl/0.05 µl*	1x
20x primer mix†	0.5–1.4 µl	0.25–0.7 µl	0.25–0.7 µM for each primer
UCP water	Variable	Variable	–
Template DNA (added at step 4)	Variable	Variable	0.01 pg – 100 ng/reaction
Total reaction volume	**20 µl**	**10 µl**	

* To be used as a 20x concentrate for high ROX cyclers (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for low ROX dye cyclers (i.e., Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems).

† A 20x primer-probe mix consists of 5 µM forward primer and 5 µM reverse primer in either TE buffer or UCP water, for a final concentration of 0.25 µM. Primers can either be premixed and added simultaneously, or added separately. The primer mix volume needs to be adjusted to achieve the desired final concentration.

4. Add template DNA (100 ng – 10 fg per reaction, depending on target abundance and sample type) to each PCR tube. Genomic DNA, cDNA, plasmid DNA, oligonucleotides and other DNA can serve as template.
5. Program the thermal cycler according to the manufacturer's instructions, using the conditions listed in either Table 2 or Table 3.
6. Place the PCR tubes or plates in the real-time cycler, and start the cycling program.

   Note: After amplification, samples can be stored at –20°C for longer storage.

Table 2. UCP SYBR® Green 2-step cycling

Step	Time	Temperature	Ramp rate
PCR initial activation	2 min	95°C	Maximal/fast mode
2-step cycling			
Denaturation	10 s	95°C	Maximal/fast mode
Annealing/Extension	10 s*	60°C	Maximal/fast mode
Number of cycles	35–45		
Melting curve analysis			

* Use this step also for data acquisition. If your cycler does not accept this short time for data acquisition, use the shortest acceptable time.

For primer sets that do not have an appropriate annealing temperature or for degenerated or long-fragment assays, we recommend using the 3-step cycling outlined below.

Table 3. UCP SYBR® Green 3-step cycling, e.g., for Earth Microbiome primer

Step	Time	Temperature	Ramp rate
Initial PCR activation	2 min	95°C	Maximal/fast mode
3-step cycling			
Denaturation	10 s	95°C	Maximal/fast mode
Annealing	20 s	50°C	Maximal/fast mode
Extension	30 s*	72°C	Maximal/fast mode; data acquisition
Number of cycles	35–45		
Melting curve analysis			

* Use this step also for data acquisition. If your cycler does not accept this short time for data acquisition, use the shortest acceptable time.

7. Perform a melting curve analysis of the PCR products.

   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.

   Optional: Check the specificity of PCR products by doing agarose gel electrophoresis.

Revision History

Document	Changes	Date
HB-2661-001	Initial release.	May 2019

Scan QR code for handbook.

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