Quick-Start Protocol May 2019

UCP Probe PCR Kit

The UCP Probe PCR Kit (cat. nos. 208212 and 208214) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. UCP Probe 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 Probe PCR Kit Handbook*: **www.qiagen.com/HB-2666**
- 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 TaqMan® probes in a singleplex or duplex reaction 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: Applied Biosystems 7000, 7300, 7900, and StepOne® Real-Time PCR Systems

- The dye in UCP Yellow Template Dilution Buffer allows tracking of pipetted samples. When this buffer is added to the blue UCP Probe PCR Master Mix, the color of the Master Mix changes from blue to green. The use of this buffer is optional. It is provided as 100x concentrate and should be diluted (using water or Tris buffer) to obtain a final concentration of 1x within samples.* The buffer does not affect sample stability or qPCR.
- For the highest efficiency in real-time PCR using TaqMan probes, amplicons should ideally be 60–150 bp in length.
- Before performing duplex analyses, choose suitable combinations of reporter dyes and quenchers that are compatible with duplex analysis using the detection optics of your real-time cycler.
- 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. For targets not suited for 60°C annealing/extension, a 3-step protocol might be applicable.
- The PCR must start with an initial incubation step of 2 min at 95°C to activate the hot-start DNA polymerase.
- For ease of use, we recommend preparing a 20x primer-probe mix containing target-specific primers and probe for each of your targets. A 20x primer-probe mix consists of 6 µM for each primer and 2 µM for each probe in UCP water. Ideal concentrations may vary, depending on the assay used.

1. Thaw UCP Probe 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.

* 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.

Table 1. Reaction setup for UCP Probe PCR Kit

Component	Volume/reaction		Final concentration
	96-well block, Rotor-Gene	384-well block	
Reaction mix			
2x UCP Probe 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-probe mix 1†	1 µl	0.5 µl	0.3 µM forward primer 1 0.3 µM reverse primer 1 0.1 µM TaqMan probe 1
20x primer-probe mix 2†	1 µl	0.5 µl	0.3 µM forward primer 2 0.3 µM reverse primer 2 0.1 µM TaqMan probe 2
UCP water	Variable	Variable	–
Template DNA (added at step 4)	Variable	Variable	≤100 ng/reaction
Total reaction volume	**20 µl**	**10 µl**	**–**

* To be used as a 20x concentrate for high ROX cyclers (i.e., 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 6 µM for each primer and 2 µM for each probe in UCP water. Ideal concentrations may vary, depending on the assay used. Primers can either be premixed and added simultaneously, or added separately.

4. Add template gDNA or cDNA (≤100 ng/reaction) to the individual PCR tubes or wells containing the reaction mix.
5. Program the real-time cycler according to Table 2 or 3. The 3-step cycling protocol is recommended for challenging samples.
   Note: Data acquisition should be performed during the combined annealing/extension step.
6. Place the PCR tubes or plates in the real-time cycler, and start the cycling program.

Table 2. Two-step cycling conditions

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

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

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

Table 3. Three-step cycling conditions, for longer or difficult amplicons

Step	Time	Temperature	Ramp rate
PCR initial heat activation	2 min	95°C	Maximal/fast mode
3-step cycling			
Denaturation	5 s	95°C	Maximal/fast mode
Annealing	15 s	50–60°C	Maximal/fast mode
Extension	20 s*	72°C	
Number of cycles	40†		

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

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

Revision History

Document	Changes	Date
HB-2660-001	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.