January 2019

Quick-Start Protocol

UCP HiFidelity PCR Kit

The UCP HiFidelity PCR Kit (cat. nos. 202742 and 202744) should be stored immediately upon receipt in a constant-temperature freezer at –30 to –15°C. The UCP HiFidelity PCR Master Mix can also be stored at 2–8°C for up to 6 months or the expiration 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 HiFidelity PCR Kit Handbook*: **www.qiagen.com/HB-2617**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- DNA polymerase in the UCP master mix requires 30 s heat activation at 98°C.
- It is not necessary to keep PCR tubes on ice, because nonspecific DNA synthesis cannot occur at room temperature, due to the inactive state of DNA polymerase.
- UCP HiFidelity PCR Kits are designed to be used with a final primer concentration of 0.25 µM for each primer. For ease of use, we recommend preparing a 20x primer mix containing target-specific primers. A 20x primer mix consists of 5 µM forward primer and 5 µM reverse primer in UCP water or TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primer solutions.
- The blue and orange dyes in the UCP template tracer and in the UCP master mix tracer, respectively, allow tracking of pipetted samples during the PCR setup. When the blue template is added to the orange PCR master mix, the color changes to green. The use of these tracers is optional. Both tracers affect neither sample stability nor PCR performance.

- The blue UCP template tracer is provided as a 25x concentrate and should be diluted to obtain a 1x final concentration in the sample.* To generate a template dilution series, dilute the 25x concentrate (using template and UCP water or respective diluent) to obtain a final concentration of 1x Template Tracer.
- The orange UCP master mix tracer is provided as a 125x concentrate and can be added either to the reaction setup (Table 1) to obtain a 1x final concentration or directly to the Master Mix vial† for long-term storage.
- Reactions can be directly loaded onto agarose gels after cycling. Each tracer dye allows monitoring of the loading process and efficient tracking of the subsequent electrophoresis. The dyes run at about 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.

Protocol

1. Thaw UCP HiFidelity PCR Master Mix, template DNA or cDNA, primer solutions, UCP PCR water, UCP template tracer (optional) and UCP master mix tracer (optional). Mix thoroughly before use.
2. For applications using generic 16S or 18S assays, appropriate measures should be taken to prevent contamination of the master mix or other components during use. These measures should include, but are not limited to, use of dedicated pipettes and tips on a UV workbench. UV irradiation of surfaces and pipettes should be performed prior to setup. Please refer to standard publications for further details.
3. Prepare a reaction mix according to Table 1. The reaction mix contains all the components except the template DNA. Prepare a volume of reaction mix 10% greater than required for the total number of reactions to be performed. It is not necessary to keep samples on ice during reaction setup or while programming the cycler.

   Note: A negative control (without template) should be included in every experiment.

* Example: Add 0.2 µl of the blue UCP Template Tracer (25x) to 5 µl sample before use. If pipetting volumes are too small to handle, the Template Tracer can be prediluted using DNA-free water. In this example, 2 µl of 1:10 prediluted Template Tracer can be added.

† Example: Add 10 µl of the UCP Master Mix Tracer (125x) to 1 tube (1.3 ml) UCP HiFidelity PCR Master Mix (2x). Since the amount of Master Mix tracer added is very small, the concentration of the Master Mix will not be changed and the UCP HiFidelity PCR Master Mix can be used as indicated in the protocol.

Table 1. Reaction setup for UCP HiFidelity PCR Kit

Component	Volume/reaction	Final concentration
UCP HiFidelity PCR Master Mix, 2x	12.5 µl	1x
20x primer mix*	1.25 µl	0.25 µM for each primer
UCP water	Variable	–
Optional: UCP master mix tracer,125x	0.1 µl	1x
Template DNA (added at step 4)	Variable	0.01 pg – 1 µg/reaction
Total reaction volume	**25 µl**†	

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

† For PCR in a 384-well plate, we recommend a final reaction volume of 15 µl. Reduce pipetting volumes accordingly.

4. Mix the reaction gently but thoroughly by pipetting up and down a few times or by briefly vortexing. Dispense into PCR tubes or the wells of a PCR plate.
5. Add template DNA (1 µg – 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. Program the thermal cycler according to the manufacturer's instructions, using the conditions listed in Table 2 and Table 3.
6. Place the PCR tubes or plates in the thermal cycler and start the PCR program.

   Note: After amplification, samples can be stored at –30 to –15°C for longer storage.

Table 2. UCP HiFidelity PCR Kit standard cycling conditions for amplicons ≤1 kb

Step	Time	Temperature	Comment
Initial PCR activation	30 s	98°C	This heating step activates the DNA polymerase.
3-step cycling			
Denaturation	10 s	98°C	Do not exceed this temperature.
Annealing	10 s	61°C	Approximately 3°C above T_m of primers.
Extension	15 s	72°C	For PCR products ≤1000 bp. For bigger amplicons, an extension time of 15–30 s/kb shall be used.
Final extension	2 min	72°C	
Number of cycles	≤40		The optimal cycle number depends on the amount of template and the abundance of the target.

This protocol is recommended for long amplicons up to 9 kb.

Table 3. UCP HiFidelity PCR cycling conditions for long amplicons

Step	Time	Temperature	Comment
Initial PCR activation	30 s	98°C	This heating step activates the DNA polymerase.
3-step cycling			
Denaturation	10 s	98°C	Do not exceed this temperature.
Annealing	10 s	61°C	Approximately 3°C above T_m of primers.
Extension	5 min	72°C	This protocol will work for amplicons of 9 kb. For shorter amplicons, 15–30 s/kb might be used as extension time.
Final extension	2 min	72°C	
Number of cycles	≤40		The optimal cycle number depends on the amount of template and the abundance of the target. However, for 16S, the number chosen should be as low as possible.

Revision History

Document	Changes	Date
HB-2575-002	Corrected the number of the related handbook in Further Information. Inserted a second footnote in Table 1. Modified comment related to annealing step in tables 2 and 3. Changed storage temperature info in protocol step 6, from "–20°C" to "–30 to –15°C". Changed "40" to "≤40" in Table 3, Number of cycles. Added Bio-Rad license notice.	January 2019

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