Quick-Start Protocol March 2018

# UCP® Multiplex PCR Kit

The UCP Multiplex PCR Kit (cat. nos. 206742, 206744) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. The UCP Multiplex Master Mix can also be stored at 2–8°C for up to 6 months, depending on the expiration date. 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 Multiplex PCR Kit Handbook*: **www.qiagen.com/HB-2532**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- DNA Polymerase in the UCP Multiplex PCR Master Mix requires a heat-activation step of 2 min at 95°C.
- It is not necessary to keep PCR tubes on ice, as non-specific DNA synthesis cannot occur at room temperature, due to the inactive state of DNA Polymerase.
- UCP Multiplex PCR Kits are designed to be used with a final primer concentration of 0.25 µM. For high-grade multiplexing approaches, the amount may need to be adapted. 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 and probe 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 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 either be added to the reaction setup (Table 1) to obtain a 1x final concentration or it can be added directly to the Master Mix vial† for long-term storage.
- Reactions can be loaded onto agarose gels directly after cycling. Each tracer dye allows monitoring of the loading process and efficient tracking during electrophoresis. The dyes run at about 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.

## Protocol

1. Thaw UCP Multiplex 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. For multiplex reactions, we recommend to prepare a combined primer mix prior to PCR setup.
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, besides 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.
4. Mix the reaction mix gently but thoroughly, by pipetting up and down a few times, or by vortexing briefly. Dispense into PCR tubes or wells of a PCR plate.

* 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 pre-diluted using DNA-free water. In this example, 2 µl of 1:10 pre-diluted Template Tracer can be added.

† Example: Add 4 µl of the UCP Master Mix Tracer (125x) to 1 tube (500µl) UCP Multiplex PCR Master Mix (4x). Since the amount of Master Mix tracer added is very small, the concentration of the Master Mix will not be changed and the UCP Multiplex PCR Master Mix can be used as indicated in the protocol.

**Table 1. Reaction setup for UCP Multiplex PCR Master Mix Kit**

| Component | Volume/reaction | Final concentration |
|---|---|---|
| 4x UCP Multiplex PCR Master Mix | 5 µl | 1x |
| 20x Primer Mix* | 1 µl | 0.25 µM for each primer |
| UCP water | Variable | – |
| Optional: UCP Master Mix Tracer,125x | 0.04 µl | 1x |
| **Template DNA (added at step 4)** | Variable | 0.01 pg – 1 µg/reaction |
| **Total reaction volume** | **20 µl** | |

* A 20x primer–probe mix consists of 5 µM forward primer and 5 µM reverse primer in TE buffer, or UCP water, for each target. Primers can either be pre-mixed 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.

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 Tables 2 and 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 –20°C for longer storage.
7. We have evaluated several specialized protocols and particular hints. For details, please refer to the *UCP Multiplex PCR Kit Handbook*.

**Table 2. UCP Multiplex PCR Kit cycling conditions for multiplex reactions**

| Step | Time | Temperature | Comment |
|---|---|---|---|
| Initial PCR activation | 2 min | 95°C | This heating step activates the DNA Polymerase. |
| 3-step cycling | | | |
| Denaturation | 5 s | 95°C | Do not exceed this temperature. |
| Annealing | 15 s | 55°C | Approximately 5°C below $T_m$ of primers. |
| Extension | 30 s | 72°C | For PCR products up to 1000 bp, an extension time of 30 s is sufficient. For multiplex reactions of more than 10 targets, or low sample amounts, increasing to 60 s/kb may be beneficial. |
| Final extension | 5 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 qualitative 16S/18S analysis of samples using primer panels with low complexity.

**Table 3. UCP Multiplex PCR cycling conditions for amplification of 16S/18S**

| Step | Time | Temperature | Comment |
|---|---|---|---|
| Initial PCR activation | 2 min | 95°C | This heating step activates the DNA Polymerase. |
| **3-step cycling:** | | | |
| Denaturation | 10 s | 95°C | Do not exceed this temperature. |
| Annealing | 30 s | 55°C | Approximately 5°C below $T_m$ of primers. |
| Extension | 15 s | 72°C | As most 16S amplicons are <500bp this is sufficient. For longer amplicons, 30 seconds may be beneficial. |
| 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. |

Scan QR code for handbook.

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