Quick-Start Protocol February 2024

UltraRun® LongRange PCR Kit

The UltraRun LongRange PCR Kit (cat. nos. 206442 and 206444) should be stored immediately upon receipt in a constant-temperature freezer at –30°C to –15°C. The UltraRun LongRange PCR Master Mix (4x) can also be stored at 2–8°C for up to 6 months or until the expiration date printed on the kit label.

Further information

- *UltraRun LongRange PCR Kit Handbook*: **www.qiagen.com/HB-2686**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- DNA Polymerase in the UltraRun LongRange PCR Master Mix (4x) requires a heat-activation step of 3 min at 93°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.
- The UltraRun LongRange PCR Kit is designed to be used with a final primer concentration of 0.5 µM. For ease of use, we recommend preparing a 20x primer mix containing target-specific primers. A 20x primer mix consists of 10 µM forward primer and 10 µM reverse primer in TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primer solutions. Depending on the application, up to 6 primer pairs can be used for multiplexed amplification.
- The blue and orange dyes in the Template Tracer and in the 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. Neither sample stability nor PCR performance is affected by either of these tracers.

- The blue 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 water or respective diluent) to obtain a final concentration of 1x Template Tracer.
- The orange 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 loaded onto agarose gel 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 dye) or 4000 bp (blue dye) on 1% agarose gel.
- The UltraRun LongRange PCR Kit is provided with Q-Solution®, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution.

Procedure

1. Thaw UltraRun LongRange PCR Master Mix, template DNA or cDNA, primer solutions, PCR water, Template Tracer (optional), Master Mix Tracer (optional), and 5x Q-Solution (optional). Mix thoroughly before use.
2. Prepare the reaction mix according to Table 1. The reaction mix contains all the components, aside from 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.
3. Mix the reaction mix gently but thoroughly by pipetting up and down a few times or by briefly vortexing. Dispense into the PCR tubes or wells of a PCR plate.

* Example: Add 0.2 µL of the blue 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 Master Mix Tracer (125x) to 1 tube (500 µL) UltraRun 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 UltraRun PCR Master Mix can be used as indicated in the protocol.

Table 1. Reaction setup for UltraRun LongRange PCR Kit

Component	Volume/reaction	Final concentration
UltraRun Long Range PCR Master Mix, 4x	5 µL	1x
20x Primer Mix*	1 µL	0.5 µM for each primer
PCR water	Variable	–
Optional: Master Mix Tracer,125x	0.04 µL	1x
Optional: Q-Solution†,5x	4 µL	1x
Template DNA (added at step 4)	Variable	0.01 ng – 1 µg/reaction
Total reaction volume	20 µL‡	

* A 20x primer–probe mix consists of 10 µM forward primer and 10 µM reverse primer in TE buffer for each target. Primers can either be pre-mixed and added simultaneously or added separately for each target. If the concentration of the primer mix(es) differ, the respective added volume needs to be adjusted to achieve a final concentration of 0.5 µM for each primer. Up to 6 primer pairs can be multiplexed.

† For templates with GC-rich regions or complex secondary structure.

‡ For PCR in a 384-well plate, we recommend a final reaction volume of 10 µL. Reduce pipetting volumes accordingly.

4. Add template DNA (1 µg – 10 pg 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.
5. 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.

Table 2. UltraRun LongRange PCR Kit cycling conditions: standard 2-step protocol

Step	Time	Temperature	Comment
Initial PCR activation	3 min	93°C	This heating step activates the DNA Polymerase.
2-step cycling			
Denaturation	30 s	93°C	Do not exceed this temperature.
Annealing/Extension	30 s/kb	65°C*	Use an extension time of 30 s per kilobase DNA for genomic DNA targets.
Final extension	10 min	72°C	
Number of cycles	≤35		The optimal cycle number depends on the amount of template and the abundance of the target.

* Standard for primers with T_m between 58–65°C.

The 3-step cycling may be used in case the annealing temperatures are significantly differing or in case a lower annealing temperature may be beneficial.

Table 3. UltraRun LongRange PCR cycling conditions: 3-step protocol

Step	Time	Temperature	Comment
Initial PCR activation	3 min	93°C	This heating step activates the DNA Polymerase.
3-step cycling			
Denaturation	30 s	93°C	Do not exceed this temperature.
Annealing	15 s	55°C	Approximately 5°C below T_m of primers.
Extension	30 s/kb	68°C	Use an extension time of 30 s per kilobase DNA for genomic DNA targets.
Final Extension	10 min	72°C	
Number of cycles	≤35		The optimal cycle number depends on the amount of template and the abundance of the target.

Document Revision History

Date	Changes
08/2019	Initial release.
01/2020	Added section on the use of Q-Solution. Change in initial PCR activation and denaturation temperature in Tables 2 and 3 from 95°C to 93°C. Change in final extension time in Table 3 from 5 to 10 minutes.
02/2024	Updated license disclaimer.

Scan QR code for handbook.

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