Quick-Start Protocol March 2016

HotStarTaq® *Plus* Master Mix Kit

The HotStarTaq *Plus* PCR Master Mix Kit (cat. nos. 203643, 203645 and 203646), including buffers and reagents, should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- *HotStarTaq* Plus *PCR Handbook*: **www.qiagen.com/HB-0450**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- HotStarTaq *Plus* DNA Polymerase requires a heat-activation step of 5 min at 95°C (see step 5).
- It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of HotStarTaq *Plus* DNA Polymerase.
- HotStarTaq *Plus* Master Mix provides a final concentration of 1.5 mM $MgCl_2$ in the reaction mix, which will give satisfactory results in most cases. However, in some cases reactions may be improved by increasing the final Mg^{2+} concentration. If a higher Mg^{2+} concentration is required, prepare a stock solution containing 25 mM $MgCl_2$ and add the appropriate volume to the reaction mix as described in the *HotStarTaq* Plus *PCR Handbook*.
- A No Template Control (NTC) should always be included.
- HotStarTaq *Plus* DNA Master Mix is provided with CoralLoad® Concentrate, which contains a gel-loading reagent and gel-tracking dyes.
- CoralLoad Concentrate must not be used in capillary sequencers.

1. Thaw primer solutions, template nucleic acid and CoralLoad Concentrate (if required). Mix thoroughly before use.
2. Thaw the HotStarTaq *Plus* Master Mix and mix by vortexing briefly to avoid localized differences in salt concentration. Dispense 10 µl into each PCR tube according to Table 1.
3. Add the appropriate volume of diluted primer mix to the PCR tubes containing HotStarTaq *Plus* Master Mix according to Table 1.

Table 1. Reaction setup using HotStarTaq *Plus* Master Mix

Component	Volume/reaction	Final concentration
HotStarTaq *Plus* Master Mix, 2x	10 µl	1 unit HotStarTaq *Plus* DNA Polymerase 1x PCR Buffer* 200 µM of each dNTP
Diluted primer mix		
Primer A	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
Optional: CoralLoad Concentrate, 10x	2 µl	1x
RNase-free water	Variable	–
Template DNA (added at step 4)	Variable	<200 ng/reaction
Total reaction volume	20 µl†	

* Contains 1.5 mM $MgCl_2$.

† If using different reaction volumes, adjust the amount of each component accordingly.

4. Add template DNA (<200 ng/20 µl reaction) to the individual PCR tubes containing the reaction mix according to Table 1. The volume added should not exceed 10% of the final PCR volume. For RT-PCR, add an aliquot from the reverse transcriptase reaction. This should not exceed 10% of the final PCR volume.
5. Program the thermal cycler according to the manufacturer's instructions.

   Note: Each PCR program must start with an initial heat activation step at 95°C for 5 min. Do not exceed the 5 min activation time. A typical PCR cycling program is outlined in Table 2.

Table 2. Optimized cycling conditions

Step	Time	Temperature	Comment
Initial heat activation	5 min	95°C	Activates HotStarTaq *Plus* DNA Polymerase.
3-step cycling:			
Denaturation	0.5–1 min	94°C	
Annealing	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers.
Extension	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles	25–35		
Final extension	10 min	72°C	

6. Place the PCR tubes in the thermal cycler and start the cycling program.

   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

7. When using CoralLoad Concentrate, the PCR products can be directly loaded onto an agarose gel without prior addition of a PCR loading buffer and gel-tracking dyes. Refer to Table 3 to identify the gel-tracking dyes present in CoralLoad Concentrate according to migration distance in different percentage agarose gels.

   Note: Due to the high viscosity of the solution, apply the solution slowly into the wells of the agarose gel.

Table 3. Migration distance of gel-tracking dyes in CoralLoad Concentrate

% TAE (TBE) agarose gel	Red dye	Orange dye
0.8	500 (270) bp	~80 (<10) bp
1.0	300 (220) bp	~40 (<10) bp
1.5	250 (120) bp	~20 (<10) bp
2.0	100 (110) bp	<10 (<10) bp
3.0	50 (100) bp	<10 (<10) bp

Scan QR code for handbook.

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