Quick-Start Protocol March 2016

HotStarTaq® Master Mix Kit

The HotStarTaq Master Mix Kit (cat. nos. 203446, 203443 and 203445), including buffers and reagents, should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- *HotStarTaq PCR Handbook*: **www.qiagen.com/HB-0452**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- HotStarTaq DNA Polymerase requires a heat-activation step of 15 min at 95°C (see step 6).
- 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 DNA Polymerase.
- HotStarTaq 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 PCR Handbook*.
- A No Template Control (NTC) should always be included.

1. Thaw primer solutions and template nucleic acid. Mix thoroughly before use.
2. Thaw the HotStarTaq Master Mix and mix by vortexing briefly to avoid localized differences in salt concentration.
3. Prepare a reaction mix according to Table 1.

   Note: The reaction mix typically contains all the components required for PCR except the template DNA. Prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.

Table 1. Reaction setup using HotStarTaq Master Mix

Component	Volume/reaction	Final concentration
Reaction mix		
HotStarTaq Master Mix, 2x	25 µl	2.5 units HotStarTaq DNA Polymerase 1x PCR Buffer* 200 µM of each dNTP
10x primer mix (2 µM of each primer)	5 µl	0.2 µM† of each primer
RNase-free water	Variable	–
Template DNA (added at step 5)	Variable	<1 µg/reaction
Total reaction volume	50 µl	

* Contains 1.5 mM $MgCl_2$.

† 0.2 µM is suitable for most PCR systems. Alternatively, perform a series of reactions using 0.1 µM to 0.5 µM of each primer to determine the optimal primer concentration.

4. Mix the reaction mix gently but thoroughly, for example by pipetting up and down a few times. Dispense appropriate volumes into PCR tubes or the wells of a PCR plate.
5. Add template DNA (<1 µg/50 µl reaction) to the individual PCR tubes or wells containing the reaction mix (see Table 1). For RT-PCR, add an aliquot from the reverse transcriptase reaction. This should not exceed 10% of the final PCR volume.
6. 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 15 min. A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

7. 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 –30 to –15°C for longer storage.

Table 2. Optimized cycling conditions

Step	Time	Temperature	Comment
Initial heat activation	15 min	95°C	Activates HotStarTaq 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	

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