Quick-Start Protocol March 2016

Taq PCR Master Mix Kit

The *Taq* PCR Master Mix Kit (cat. nos. 201443 and 201445), including buffers and reagents, should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- Taq *PCR Handbook*: **www.qiagen.com/HB-0455**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- *Taq* PCR 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 Taq *PCR Handbook*.
- A No Template Control (NTC) should always be included.
- It is recommended that the PCR tubes be kept on ice until they are placed in the thermal cycler.

1. Thaw primer solutions and template nucleic acid. Keep on ice after complete thawing, and mix thoroughly before use.
2. Thaw *Taq* PCR 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 *Taq* PCR Master Mix

Component	Volume/reaction	Final concentration
Reaction mix *Taq* PCR Master Mix, 2x	50 µl	2.5 units *Taq* DNA Polymerase 1x QIAGEN PCR Buffer* 200 µM of each dNTP
10x primer mix (2 µM of each primer)	10 µl	0.2 µM† of each primer
RNAse-free water (provided)	Variable	–
Template DNA (added at step 5)	Variable	≤1 µg/reaction
Total reaction volume	100 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.

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

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/reaction) to the individual PCR tubes or wells containing the reaction mix. For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of the final PCR volume.
6. Program the thermal cycler according to the manufacturer's instructions. A typical PCR cycling program is outlined in Table 2.

Table 2. Optimized cycling conditions

Step	Time	Temperature	Comment
Initial denaturation	3 min	94°C	
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	

7. For a simplified hot start, proceed as described in step 7a. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.

   7a. Simplified hot start: Start the PCR program. Once the thermal cycler has reached 94°C, place the PCR tubes in the thermal cycler. In many cases, this simplified hot start improves the specificity of the PCR. For highly specific and convenient hot-start PCR, use HotStarTaq® *Plus* DNA Polymerase.

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

Scan QR code for handbook.

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