Quick-Start Protocol March 2016

Taq DNA Polymerase and *Taq* PCR Core Kit

Taq DNA Polymerase (cat. nos. 201203, 201205, 201207 and 2012099) and the *Taq* PCR Core Kit (cat. nos. 201223 and 201225), 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* DNA Polymerase and the *Taq* PCR Core Kit are provided with Q-Solution®, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich by modifying the melting behavior of DNA. When using Q-Solution for the first time for a particular primer–template pair, always perform parallel reactions with and without Q-Solution.
- *Taq* DNA Polymerase and *Taq* PCR Core Kits are provided with CoralLoad® PCR Buffer, which contains a gel-loading reagent and gel-tracking dyes.
- The PCR Buffer and CoralLoad PCR Buffer provide a final concentration of 1.5 mM $MgCl_2$ in the final 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, add the appropriate volume of 25 mM $MgCl_2$ to the reaction mix as described in the Taq *PCR Handbook*.
- High-quality, PCR-grade dNTP Mix (10 mM) is available separately from QIAGEN (cat. no. 201900) if needed.

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

1. Thaw 10x CoralLoad PCR Buffer or 10x PCR Buffer, dNTP mix, primer solutions, Q-Solution (if required) and 25 mM $MgCl_2$ (if required) at room temperature or on ice. Keep on ice after complete thawing and mix thoroughly before use to avoid localized differences in salt concentration.
2. Prepare a reaction mix according to Table 1. The reaction mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of reaction mix 10% greater than that required for the total number of PCR assays to be performed.

Table 1. Reaction setup using *Taq* DNA Polymerase

Component	Volume/reaction	Final concentration
Reaction mix		
10x PCR Buffer* or **Optional**: 10x CoralLoad PCR Buffer*	10 µl	1x
dNTP mix (10 mM of each)	2 µl	200 µM of each dNTP
Primer A	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
Taq DNA Polymerase	0.5 µl	2.5 units/reaction
RNAse-free water	Variable	–
Optional: 5x Q-Solution†	20 µl	1x
Template DNA (added at step 4)	Variable	≤1 µg/reaction
Total reaction volume	100 µl‡	

* Contains15 mM $MgCl_2$.

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

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

3. Mix the reaction mix gently but thoroughly, for example by pipetting up and down a few times. Dispense appropriate volumes into PCR tubes.
4. Add template DNA (≤1 µg/reaction) to the individual PCR tubes 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.
5. 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 for *Taq* DNA Polymerase

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	

6. For a simplified hot start, proceed as described in step 6a. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.

   6a. 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.
7. When using CoralLoad PCR Buffer, 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 PCR Buffer according to migration distance in different percentage agarose gels.

Table 3. Migration distance of gel tracking dyes in CoralLoad PCR Buffer

% 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

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