Quick-Start Protocol March 2016

# HotStarTaq® *Plus* DNA Polymerase

HotStarTaq *Plus* DNA Polymerase (cat. nos. 203601, 203603, 203605, 203607 and 203609), 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).
- The PCR Buffer 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, add the appropriate volume of 25 mM $MgCl_2$ to the reaction mix as described in the *HotStarTaq* Plus *PCR Handbook*.
- If required, prepare a dNTP mix containing 10 mM of each dNTP. Store this mix in aliquots at –20°C. High-quality, PCR-grade dNTP mix (10 mM) is available from QIAGEN (cat. no. 201900).
- HotStarTaq *Plus* DNA Polymerase is 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.

- HotStarTaq *Plus* DNA Polymerase is provided with CoralLoad® PCR Buffer, which contains a gel-loading reagent and gel-tracking dyes.
- CoralLoad PCR Buffer must not be used in capillary sequencers.
- 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.
- A No Template Control (NTC) should always be included.

1. Thaw 10x CoralLoad PCR Buffer or 10x PCR Buffer, dNTP mix, primer solutions and 25 mM $MgCl_2$ (if required). Mix the solutions 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 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 HotStarTaq *Plus* 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 |
| HotStarTaq *Plus* DNA Polymerase | 0.5 µl | 2.5 units/reaction |
| **Optional**: 5x Q-Solution† | 20 µl | 1x |
| **Template DNA** (added at step 4) | Variable | ≤1 µg/reaction |
| **Total reaction volume** | 100 µl‡ | |

* Contains 15 mM $MgCl_2$.

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

‡ If using different reaction volumes, adjust the volume 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/100 µl reaction) to the individual PCR tubes containing the reaction mix. 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. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

**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 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.

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