HotStarTaq DNA Polymerase

HotStarTaq DNA Polymerase, including buffers and reagents, should be stored immediately upon receipt at -30 to -15°C in a constant-temperature freezer.

Notes before starting

- HotStarTaq DNA Polymerase requires a heat-activation step of 15 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 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 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.
- 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.
- A No Template Control (NTC) should always be included.

Operation steps

1.Thaw 10x PCR Buffer, dNTP mix, primer solutions, Q-Solution (if required) and 25 mM $MgCl^2$ (if required). 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 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.

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 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.

6. Place the PCR tubes in the thermal cycler and start the cycling program. Note: After amplification, samples can be stored overnight at 28°C, or at -20°C for longer storage.

Table 1. Reaction setup using HotStarTaq DNA Polymerase

Component	**Volume/reaction**	**Final concentration**
Reaction mix		
10x PCR Buffer*	10 µl	1x
Optional: 5x Q-Solution†	20 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 DNA Polymerase	0.5 µl	2.5 units/reaction
Distilled water	Variable	–
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.

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	