Quick-Start Protocol March 2016

HotStar HiFidelity Polymerase Kit

The HotStar HiFidelity Polymerase Kit (cat. nos. 202602 and 202605) should be stored immediately upon receipt at –30 to –15°C until the expiration date.

Further information

- *HotStar HiFidelity PCR Handbook*: **www.qiagen.com/HB-0451**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Refer to the handbook for amplification using HotStar HiFidelity DNA Polymerase and amplification of long PCR products.
- Use a final primer concentration of 1 µM for each primer.
- ■ denotes amplification of PCR products ≤2kb, ▲ denotes amplification of PCR products 2–5 kb.
- When using Q-Solution® it is important to perform parallel amplification reactions without Q-Solution.

1. Thaw 5x HotStar HiFidelity PCR Buffer, primer solutions, 5x Q-Solution (optional) and 25 mM $MgSO_4$ (if required). Mix the solutions completely before use.
2. Prepare a reaction mix according to Table 1.
3. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes.
4. Add template DNA to the individual tubes containing the reaction mix according to Table 2.
5. Program the thermal cycler according to the manufacturer's instructions. Each PCR program must start with an initial heat-activation step at 95°C for 5 min.
6. Place the PCR tubes in the thermal cycler and start the cycling program.

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

Table 1. PCR components (reaction mix and template DNA)

Component	Volume/reaction	Final concentration
5x HotStar HiFidelity PCR Buffer (contains dNTPs)*	10 µl	1x
Optional: 5x Q-Solution	10 µl	1x
Primer A	Variable; see Table 2	1 µM
Primer B	Variable; see Table 2	1 µM
HotStar HiFidelity DNA Polymerase (2.5 units/µl)	■ 1 µl,† ▲ 2 µl†	■ 2.5 units, ▲ 5 units
RNase-free water	Variable; see Table 2	–
Template DNA, added at step 4	Variable; see Table 2	Variable; see Table 2
Total volume	50 µl	–

* Contains optimized concentration of dNTPs and 7.5 mM $MgSO_4$. The Mg^{2+} concentration provided gives successful PCR results in most cases.

† Dependent on expected PCR product length. In general, use ■ 1 µl enzyme when amplifying PCR products ■ ≤2kb and ▲ 2 µl enzyme when amplifying PCR products ▲ 2–5 kb.

Table 2. Optimal amounts of starting template from different origins

Starting template	Optimal range
Human genomic DNA	1 ng – 200 ng
cDNA‡	10 ng – 100 ng
Bacterial DNA	10 pg – 10 ng
PCR fragment (1 kb DNA)	10 fg – 1 ng
Plasmid DNA	0.1 ng – 50 ng

‡ Optimal starting template amount depends on the abundance of the respective molecule in your sample. The lower value refers to highly abundant transcripts and the upper values to low abundant transcripts. To amplify long cDNA species, it is strongly recommended to use cDNA generated using oligo-dT primers only.

Scan QR code for handbook.

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