

March 2010

Important Note

Dear EasyXpress® Linear Template Kit Plus user,

We wish to inform you that the ProofStart® DNA Polymerase provided in the EasyXpress Linear Template Kit Plus (20) (orange screw cap) is replaced by HotStar HiFidelity DNA Polymerase (red screw cap). The new polymerase ensures superior, high-fidelity PCR results and offers advantages such as:

- Maximum sensitivity due to a novel buffer additive
- Increased convenience as dNTPs are already included in the buffer

Addition of dNTPs to the PCR reaction is no longer required, as the HotStar HiFidelity PCR Buffer contains dNTPs at an optimized concentration of 1.5 mM. Furthermore, the buffer contains Factor SB to prevent degradation of primers and template during PCR setup, ensuring highly sensitive and reliable high-fidelity PCR results. The new and improved reaction composition is outlined in Table 1.

Greater time savings are also achieved as the denaturation step of the PCR cycle is now shortened to 15 seconds. See Table 2 for details of the new cycling protocol.



Table 1. Reaction composition*

Component	Volume reaction	Final concentration
Master Mix 5x HotStar HiFidelity PCR Buffer (contains dNTPs) [†]	5 μ l	1x
Sense primer	0.75–2.5 μ l	0.3–1 μ M
Antisense primer	0.75–2.5 μ l	0.3–1 μ M
HotStar HiFidelity DNA Polymerase (2.5 units/ μ l) [‡]	● 0.5 μ l ▲ 1 μ l	● 1.25 units ▲ 2.5 units
RNase-free water	Variable	–
Template		
Template DNA (added in step 4)	Variable	50–500 ng genomic DNA 1–100 ng cDNA 0.5–5.0 ng plasmid DNA
Total volume	25 μ l	–

* Please use values listed in this table for reaction setup instead of those listed in Tables 3 and 4 (pages 19 and 21, respectively), in the *EasyXpress Linear Template Kit Plus Handbook*.

[†] Contains optimized concentration of dNTPs, 7.5 mM MgSO₄, and Factor SB.

[‡] Dependent on expected PCR product length. In general, use ● 0.5 μ l enzyme when amplifying PCR products <2 kb and ▲ 1 μ l enzyme when amplifying PCR products >2 kb.

Table 2. Optimized cycling protocol

	Old cycling protocol using ProofStart DNA Polymerase		New cycling protocol using HotStar HiFidelity Polymerase	
Initial activation step	5 min	95°C	5 min	95°C
3-step cycling				
Denaturation	1 min	94°C	15 s	94°C
Annealing	1 min	50°C	1 min	50°C
Extension	1 min/kb (PCR products of 1–2 kb)	72°C	1 min/kb (PCR products of 1–2 kb)	72°C
	2 min/kb (PCR products >2kb)	72°C	2 min/kb (PCR products >2kb)	68°C
Number of cycles	30		30–45	
Final extension	7 min	72°C	10 min	72°C

If you have any questions, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover of our handbooks or visit www.qiagen.com).

Best regards,

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