LongAmp® Hot Start Taq DNA Polymerase

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M0534S

500 units **2,500 U/ml** **Lot: 0031212**
RECOMBINANT **Store at –20°C** **Exp: 12/14**

Description: LongAmp Hot Start *Taq* DNA Polymerase is a unique blend of aptamer-based Hot Start *Taq* and Deep Vent$_R$™ DNA Polymerases. The aptamer-based inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits assembly of PCR reactions at room temperature. An advantage of the aptamer-based hot start mechanism is that it does not require a separate high temperature incubation step to activate the enzyme. The 3´→5´ exonuclease activity of Deep Vent$_R$ DNA Polymerase increases the fidelity and robust amplification of Hot Start *Taq* DNA Polymerase (1). LongAmp Hot Start *Taq* DNA Polymerase offers two-fold higher fidelity than Hot Start *Taq* DNA Polymerase alone. A wide range of PCR products can be generated; up to 30 kb from lambda or human genomic DNA.

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1 and an *E. coli* strain that carries the Deep Vent$_R$ DNA Polymerase gene from *Pyrococcus* species GB-D.

Applications:
- High-specificity Long Range PCR

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween® 20, 0.5% IGEPAL® CA-630 and 50% glycerol.

Reagents Supplied with Enzyme:
5X LongAmp *Taq* Reaction Buffer

Reaction Conditions: 1X LongAmp *Taq* Reaction Buffer, DNA template, primers, 300 µM dNTPs (not included) and 1–5 units of LongAmp Hot Start *Taq* DNA Polymerase in a total reaction volume of 50 µl.

1X LongAmp Taq **Reaction Buffer:**
60 mM Tris-SO_4 (pH 9.0 @ 25°C)
20 mM $(NH_4)_2SO_4$
2 mM $MgSO_4$
3% glycerol
0.06% IGEPAL CA-630
0.05% Tween 20

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.

Unit Assay Conditions: 1X ThermoPol® Reaction Buffer, 200 µM dNTPs including [^{3}H]-dTTP and 200 µg/ml activated Calf Thymus DNA.

Heat Inactivation: No

Quality Control Assays

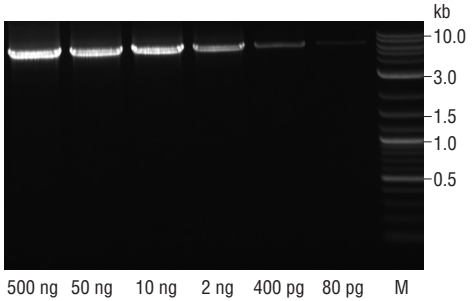
Long Amplicon PCR (Lambda DNA): 28 cycles of PCR amplification in a 25 µl reaction containing 1 ng of Lambda DNA with 2.5 units of LongAmp Hot Start *Taq* DNA Polymerase in the presence of 300 µM dNTPs and 0.4 µM primers in LongAmp *Taq* Reaction Buffer results in the expected 20 and 30 kb products.

Long Amplicon PCR (Genomic DNA): 28 cycles of PCR amplification in a 25 µl reaction containing 500 ng of human genomic DNA with 2.5 units of LongAmp Hot Start *Taq* DNA Polymerase in the presence of 300 µM dNTPs and 0.4 µM primers in LongAmp *Taq* Reaction Buffer results in the expected 20 and 30 kb products.

High Sensitivity PCR: 35 cycles of PCR amplification of 2 ng of human genomic DNA with 5 units of LongAmp Hot Start *Taq* DNA Polymerase in the presence of 200 µM dNTPs and 0.2 µM primers in 1X LongAmp *Taq* Reaction Buffer results in the hot start-specific expected 306 bp product after pre-incubation at room temperature for 1 hour.

Inhibition Assay: > 95% inhibition is observed after a 16 hour incubation at 25°C in a 50 µl primer extension assay containing 10 units of LongAmp Hot Start *Taq* DNA Polymerase in 1X ThermoPol Reaction Buffer with 200 µM dNTPs including [^{3}H]-dTTP and 15 nM primed M13 DNA template.

Note: Product specifications for individual components in the LongAmp Hot Start *Taq* DNA Polymerase are available separately.



Amplification of a 6 kb amplicon from varying amounts of human genomic DNA using LongAmp Hot Start Taq DNA Polymerase. Starting template amounts are indicated below the gel. Marker M is the NEB 2-Log DNA Ladder (NEB #N3200).

PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique of DNA amplification (2). *Taq* DNA Polymerase is an enzyme widely used in PCR (3). LongAmp Hot Start *Taq* DNA Polymerase allows for greater PCR sensitivity and permits room temperature PCR set-up. The following guidelines are provided to ensure successful PCR using New England Biolabs' LongAmp Hot Start *Taq* DNA Polymerase. These guidelines cover routine PCR reactions. Amplification of templates with high GC content, high secondary structure or low template concentrations may require further optimization.

Reaction Setup:

Due to the hot-start nature of the enzyme, reactions can be assembled on the bench at room temperature and transferred to a thermocycler. No separate activation step is required.

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X LongAmp *Taq* Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.75 µl	1.5 µl	300 µM
10 µM Forward Primer	1 µl	2 µl	0.4 µM (0.05–1 µM)
10 µM Reverse Primer	1 µl	2 µl	0.4 µM (0.05–1 µM)
Template DNA	variable	variable	<1,000 ng
LongAmp Hot Start *Taq* DNA Polymerase	1 µl	2 µl	5 units/50 µl PCR
Nuclease-free water	to 25 µl	to 50 µl	

Notes: Gently mix the reaction. Avoid pipetting samples containing target DNA when amplicons above 20 kb are desired. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes to a PCR machine and begin thermocycling:

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds
30 Cycles	94°C 45–65°C 65°C	10–30 seconds 15–60 seconds 50 seconds/kb
Final Extension	65°C	10 minutes
Hold	4–10°C	

General Guidelines:

1. Template:
   The quality of the DNA template is essential for long-range PCR amplification. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	Up to 15 kb	Above 15 kb
Genomic	1 ng–500 ng	10 ng–1 µg
Plasmid or Viral	1 pg–1 ng	10 pg–10 ng

   Successful amplification above 20 kb largely depends on the quality of DNA templates and the primer sequences.

2. Primers:
   Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. For amplicons larger than 20 kb, it is desirable to have primers with GC content above 50%, matched T_ms above 60°C and at least 24 nucleotides in length. The final concentration of each primer in a PCR reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

3. Mg^{++} and additives:
   Mg^{++} concentration of 1.0–2.0 mM is optimal for most PCR products generated with LongAmp Hot Start *Taq* DNA Polymerase. The final Mg^{++} concentration in 1X LongAmp *Taq* Reaction Buffer is 2 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.5 or 1.0 mM increments using $MgSO_4$ (not provided).

   Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (4) or formamide (5).

(see other side)

4. Deoxynucleotides:
The recommended final concentration of dNTPs for long-range PCR is 300 µM of each deoxynucleotide.

5. LongAmp Hot Start *Taq* DNA Polymerase concentration:
We generally recommend using LongAmp Hot Start *Taq* DNA Polymerase at a concentration of 200 units/ml (5 units/50 µl reaction). However, the optimal concentration of LongAmp Hot Start *Taq* DNA Polymerase may vary in specialized applications.

6. Denaturation:
No separate activation step is required to release the hot start inhibitor from the enzyme.

   An initial denaturation of 30 seconds at 94°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 94°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minute denaturation at 94°C is recommended.

   During thermocycling, a 10-30 second denaturation at 94 °C is recommended.

7. Annealing:
The annealing step is typically 15–60 seconds. Annealing temperature is based on the T_m of the primer pair and is typically 45–65°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated T_m. We recommend using NEB's T_m Calculator, available at www.neb.com/TmCalculator to determine appropriate annealing temperatures for PCR.

   When primers with annealing temperatures above 60°C are used, a 2-step PCR protocol is possible (see #10).

8. Extension:
The recommended extension temperature is 65°C. Extension times are generally 50 seconds per kb. A final extension of 10 minutes at 65°C is recommended.

9. Cycle Number:
Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low copy number targets.

10. 2-step PCR:
When primers with annealing temperatures above 60°C are used, a 2-step thermocycling protocol is possible.

Thermocycling Conditions for a Routine 2-Step PCR:

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds
30 Cycles	94°C	10–30 seconds
	60–65°C	50 seconds/kb
Final Extension	60–65°C	10 minutes
Hold	4–10°C	

11. PCR product:

    The majority of the PCR products generated using LongAmp Hot Start *Taq* DNA Polymerase contain dA overhangs at the 3´-end; therefore the PCR products can be ligated to the dT/dU-overhang vectors.

FAQs:

1. *What is the recommended enzyme amount when using LongAmp Hot Start?*

   In general, we recommend 5 units of LongAmp Hot Start *Taq* DNA Polymerase in a 50 µl reaction. For amplicons < 8 kb, we recommend 1-2.5 units per 50 µl reaction for higher fidelity.

2. *What is the fidelity of the LongAmp Hot Start Taq DNA Polymerase compared to Taq DNA Polymerase?*

   The LongAmp Hot Start *Taq* DNA Polymerase offers two-fold higher fidelity than *Taq*.

3. *Can the extension step be carried out at 72°C when using LongAmp Hot Start?*

   Yes, LongAmp Hot Start *Taq* DNA Polymerase can be used at 72°C. However, extension at 65–68°C is a better choice for most amplicons.

4. *What is the extension rate when using LongAmp Hot Start?*

   We recommend 50 seconds per kb for maximum yields. Extension rates such as 30 seconds per kb can be used for targets up to 4 kb using a 3-step PCR protocol. Shorter extension rates, such as 15 seconds per kb, can be used for targets up to 2 kb using a 3-step PCR protocol on a fast PCR machine.

5. *What type of DNA ends result from a primer extension reaction or a PCR using LongAmp Hot Start Taq DNA Polymerase?*

   The majority of the PCR products generated using LongAmp Hot Start *Taq* DNA Polymerase contain dA overhangs at the 3´-end; therefore, the PCR products can be ligated to dT/dU-overhang vectors.

6. *Why is the product a smear when visualized on an agarose gel?*

   When PCR conditions are not optimal, a smear or high level of background is often observed. Try one or more of the following suggestions:
   - use lower amount of enzymes
   - use 65°C for extension
   - raise annealing temperature
   - try 2-step cycling protocols

7. *Can LongAmp Hot Start Taq DNA Polymerase be used to amplify GC-rich amplicons?*

   Yes. The addition of DMSO up to 10% helps amplify GC-rich amplicons.

References:

1. Barnes, W.M. (1994) *Proc. Natl. Acad. Sci. USA,* 91, 2216–2220.
2. Saiki R.K. et al. (1985) *Science,* 230, 1350–1354.
3. Powell, L.M. et al. (1987) *Cell,* 50, 831–840.
4. Sun, Y., Hegamyer, G. and Colburn, N. (1993) *Biotechniques,* 15, 372–374.
5. Sarkar, G., Kapelner, S. and Sommer, S.S. (1990) *Nucleic Acids Res.,* 18, 7465.

Companion Products Sold Separately:

LongAmp *Taq* (Mg-free) Reaction Buffer Pack
#B0322S 6.0 ml

LongAmp *Taq* Reaction Buffer Pack
#B0323S 6.0 ml

Crimson LongAmp *Taq* Reaction Buffer Pack
#B0326S 6.0 ml

Magnesium Sulfate ($MgSO_4$) Solution
#B1003S 6.0 ml

Diluent F
#B8006S 4.0 ml

LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 Reactions
#M0533L 500 Reactions

LongAmp *Taq* PCR Kit
#E5200S 100 Reactions

LongAmp *Taq* 2X Master Mix
#M0287S 100 Reactions
#M0287L 500 Reactions

Crimson LongAmp *Taq* DNA Polymerase
#M0326S 250 units
#M0326L 1,250 units

Deoxynucleotide Solution Set
#N0446S 25 µmol of each

Deoxynucleotide Solution Mix
#N0447S 8 µmol of each
#N0447L 40 µmol of each

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