

**LongAmp®
Hot Start Taq
DNA Polymerase**



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M0534S



500 units 2,500 U/ml Lot: 0041310
RECOMBINANT Store at -20°C Exp: 10/15

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-SO₄ (pH 9.0 @ 25°C)
20 mM (NH₄)₂SO₄
2 mM MgSO₄
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 [³H]-dTTP and 200 µg/ml activated Calf Thymus DNA.

Heat Inactivation: No

Quality Control Assays

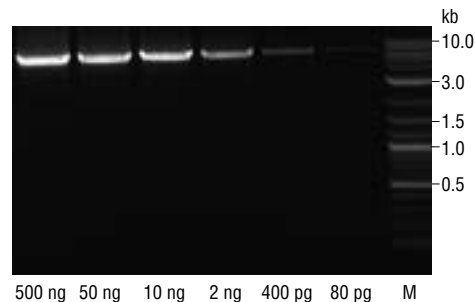
Long Amplicon PCR (Lambda DNA): 28 cycles of PCR amplification in a 25 µl reaction containing 1 ng of Lambda DNA with 2.5 units of LongAmp Hot Start Taq DNA Polymerase in the presence of 300 µM dNTPs and 0.4 µM primers in LongAmp Taq Reaction Buffer results in the expected 20 and 30 kb products.

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 [³H]-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 | 10–30 seconds |
| | 45–65°C | 15–60 seconds |
| | 65°C | 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 MgSO₄ (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 μ M of each deoxy-nucleotide.

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 μ l 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 μ l reaction. For amplicons < 8 kb, we recommend 1-2.5 units per 50 μ l 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 (MgSO₄) 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 μ mol of each

Deoxynucleotide Solution Mix #N0447S 8 μ mol of each
#N0447L 40 μ mol of each



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