

**LongAmp®
Hot Start Taq
2X Master Mix**



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M0533S



100 reactions (50 µl vol) Lot: 0111503
RECOMBINANT Store at -20°C Exp: 9/16

Description: LongAmp Hot Start Taq 2X Master Mix contains 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. The LongAmp Hot Start Taq 2X Master Mix 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. The convenient master mix formulation is supplied at a 2X concentration and contains dNTPs and Mg⁺⁺, requiring only the addition of primers and DNA template for robust amplification. 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
- High-throughput PCR

Reaction Conditions: 1X LongAmp Hot Start Taq Master Mix, DNA template and primers in a total reaction volume of 50 µl.

1X LongAmp Hot Start Taq 2X Master Mix:

- 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
- 0.3 mM dNTPs
- 125 units/ml LongAmp Hot Start Taq DNA Polymerase

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 15 nM primed M13 DNA.

Heat Inactivation: No

Quality Control Assays

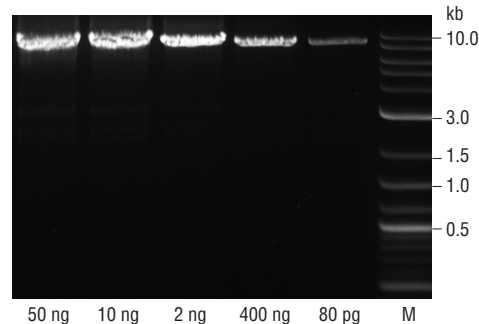
Long Amplicon PCR (Lambda DNA): 28 cycles of PCR amplification in a 25 µl reaction containing 1 ng of Lambda DNA with 1X LongAmp Hot Start Taq 2X Master Mix and 0.4 µM primers results in the expected 20 and 30 kb products.

Long Amplicon PCR (Genomic DNA): 28 cycles of PCR amplification in a 25 µl reaction containing 500 ng of human genomic DNA with 1X LongAmp Hot Start Taq 2X Master Mix and 0.4 µM primers 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 2X Master Mix are available separately.



Amplification of a 8 kb amplicon from varying amounts of Jurkat genomic DNA using LongAmp Hot Start Taq 2X Master Mix. 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 2X Master Mix. 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
LongAmp Hot Start Taq 2X Master Mix	12.5 µl	25 µl	1X
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
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⁺⁺, Deoxynucleotides and Additives:**
At a 1X concentration, LongAmp Hot Start Taq Master Mix contains 2 mM MgSO₄ and 300 µM of each dNTP in the final reaction. 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. Denaturation:
LongAmp Hot Start *Taq* DNA Polymerase does not require a separate activation step.

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.

5. 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 #8).

6. 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.
7. Cycle Number:
Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low copy number targets.

8. 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 60–65°C	10–30 seconds 50 seconds/kb
Final Extension	60–65°C	10 minutes
Hold	4–10°C	

9. 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 fidelity of the LongAmp Hot Start Taq 2X Master Mix compared to Taq DNA Polymerase?*

The LongAmp Hot Start *Taq* 2X Master Mix offers two-fold higher fidelity than *Taq*.

2. *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.

3. *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.

4. *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.

5. *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

6. *Can LongAmp Hot Start Taq 2X Master Mix 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 Hot Start *Taq* DNA Polymerase

#M0534S 100 units
#M0534L 500 units

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

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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