

LongAmp® Taq 2X Master Mix



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M0287S 021140315091

M0287S



100 reactions (50 µl vol) Lot: **0211403**
RECOMBINANT Exp: 9/15 Store at **-20°C**

Description: The LongAmp Taq 2X Master Mix combines high quality NEB recombinant Taq DNA Polymerase with a trace amount of Deep Vent_R™ DNA Polymerase. The 3'→5' exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of Taq DNA Polymerase (1). This ready-to-use mix offers two fold higher fidelity than Taq DNA Polymerase alone. A wide range of PCR products can be generated, up to 30 kb from lambda DNA or from genomic DNA (Figure 1).

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:

- Long Range PCR

Reaction Conditions: 1X LongAmp Taq Master Mix, DNA template and primers in a total reaction volume of 25–50 µl.

1X LongAmp Taq Master Mix:

60 mM Tris-SO₄ (pH 9.0 @ 25°C)
20 mM (NH₄)₂SO₄
2 mM MgSO₄
125 units/ml LongAmp Taq DNA Polymerase
0.3 mM dNTPs
3% glycerol
0.06% IGEPAL® CA-630
0.05% Tween® 20

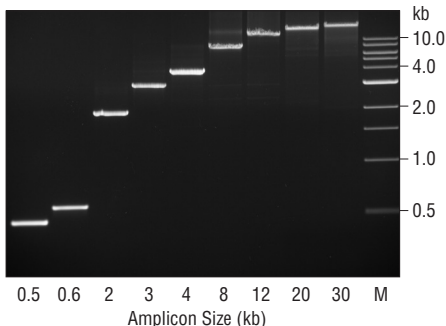
Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.

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 Controls

Long Amplicon PCR: LongAmp Taq DNA Polymerase is tested for the ability to amplify a 30 kb amplicon from lambda DNA and a 30 kb amplicon from human genomic DNA.



Amplification of specific sequences from human genomic DNA using LongAmp Taq DNA Polymerase. Amplicon sizes are indicated below the gel. Marker M is the NEB 1 kb DNA Ladder (NEB #N3232).

PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (2). Taq DNA Polymerase is an enzyme widely used in PCR (3). The following guidelines are provided to ensure successful PCR using New England Biolabs' LongAmp 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:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (94°C).

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
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)
LongAmp Taq 2X Master Mix	12.5 µl	25 µl	1X
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 from ice to a PCR machine with the block preheated to 94°C 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_m above 60°C and primers 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 1.5–2.0 mM is optimal for most PCR products generated with LongAmp Taq DNA Polymerase. The final Mg⁺⁺ concentration in 1X LongAmp Taq Master Mix 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.

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

4. **Denaturation:**
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.

(see other side)

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	10–30 seconds
	60–65°C	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 *Taq* DNA Polymerase contain dA overhangs at the 3′-end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

Notes: LongAmp *Taq* 2X Master Mix is stable for fifteen freeze-thaw cycles when stored at –20°C

LongAmp *Taq* 2X Master Mix is also stable for three months at 4°C, so for frequent use, an aliquot may be kept at 4°C.

FAQs:

1. *What is the fidelity of the LongAmp Taq DNA Polymerase compared to Taq DNA Polymerase?*

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

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

Yes, LongAmp *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?*

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 reaction using LongAmp Taq DNA Polymerase?*

The majority of the PCR products generated using LongAmp *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 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 *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* DNA Polymerase
#M0323S 500 units
#M0323L 2,500 units

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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