

LongAmp® Taq PCR Kit



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E5200S 035141216121

E5200S



100 reactions (50 µl vol) Lot: 0351412

Store at -20°C Exp: 12/16

Description: The LongAmp Taq PCR Kit features LongAmp Taq DNA Polymerase, which is a unique blend of Taq and Deep Vent_r DNA Polymerases. The 3'→5' exonuclease activity of Deep Vent_r Polymerase increases the fidelity and robust amplification of Taq DNA Polymerase (1). LongAmp Taq DNA Polymerase offers two fold higher fidelity than Taq Polymerase alone. The LongAmp Taq PCR Kit is supplied with LongAmp Taq Reaction Buffer, which is optimized to give highest PCR yields up to 30 kb. The kit can generate a wide range of PCR products, up to 30 kb from plasmid or from human genomic DNA.

Applications:

- Long Range PCR

Advantages:

- Offers two fold higher fidelity than Taq DNA Polymerase alone
- Allows for robust amplification of PCR products up to 30 kb from low complexity templates and from difficult templates, like human genomic DNA

Kit components

5X LongAmp Taq Reaction Buffer	1.5 ml
100 mM MgSO ₄	1.5 ml
2,500 units/ml LongAmp Taq DNA Polymerase	200 µl
10 mM dNTPs	200 µl
Nuclease-free water	2 x 1.5 ml

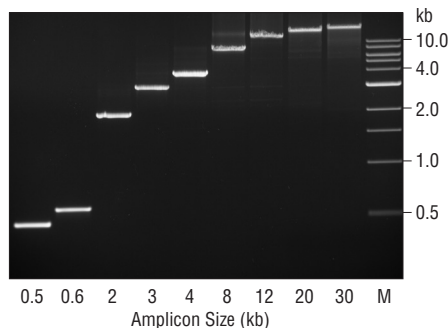
LongAmp Taq DNA Polymerase supplied in:
100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween® 20, 0.5% IGEPAL® CA-630 and 50% glycerol.

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

Quality Controls

Long Amplicon PCR: LongAmp Taq DNA Polymerase is tested for the ability to generate 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 (2). The following guidelines are provided to ensure successful PCR using New England Biolabs' LongAmp Taq DNA Polymerase. These guidelines cover routine PCR reactions. PCR 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
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)
LongAmp Taq DNA Polymerase	1 µl	2 µl	5 units/ 50 µl PCR
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 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.

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

4. **Deoxynucleotides:**
The recommended final concentration of dNTPs for long-range PCR is 300 µM of each deoxynucleotide.
5. **LongAmp Taq DNA Polymerase concentration:**
We generally recommend using LongAmp Taq DNA Polymerase at a concentration of 100 units/ml (5 units/50 µl reaction). However, the optimal concentration of LongAmp Taq DNA Polymerase may range in specialized applications.

6. **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.

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).

(see other side)

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 Taq DNA Polymerase contain dA overhangs at the 3'-end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

FAQs:

1. *What is the recommended enzyme amount when using LongAmp?*
In general, we recommend 5 units of LongAmp Taq DNA Polymerase in a 50 µl PCR reaction. For amplicons < 8 kb, we recommend 1–2.5 units per 50 µl PCR reaction for higher fidelity.
2. *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.
3. *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.

4. *What is the extension rate when using LongAmp?*
We recommend 50 seconds per kb for maximum yields. Extension rate 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 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.

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 the LongAmp kit 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
- Diluent F #B8006S 4.0 ml
- LongAmp Taq 2X Master Mix #M0287S 100 reactions #M0287L 500 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

Annex 5: Annealing temperature



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