Crimson LongAmp® *Taq* DNA Polymerase

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M0326S

250 units **2,500 U/ml** **Lot: 0101212**

RECOMBINANT **Store at –20°C** **Exp: 12/14**

Description: LongAmp *Taq* DNA Polymerase is a unique blend of *Taq* and Deep Vent$_R$™ DNA Polymerases. The 3´→ 5´ exonuclease activity of Deep Vent$_R$ DNA Polymerase increases the fidelity and robust amplification of *Taq* Polymerase (1).

Crimson LongAmp *Taq* DNA Polymerase combines the robust LongAmp *Taq* DNA Polymerase with a colored reaction buffer. Crimson LongAmp *Taq* DNA Polymerase can amplify up to 20 kb with minimal or no optimization from DNA samples of both low complexity (i.e. plasmid) and high complexity (i.e. genomic DNA). Maximum amplicon sizes are 30 kb from lambda DNA or from human genomic DNA. It offers three unique features, including a color indicator for reaction setup, direct loading of PCR product onto a gel and a tracking dye during electrophoresis.

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
- Colony PCR
- High Throughput 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 Crimson LongAmp *Taq* Reaction Buffer

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

1X Crimson 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
Acid Red

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: Crimson 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.

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' Crimson LongAmp *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:

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 Crimson 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)
Crimson 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 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_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 Crimson LongAmp *Taq* DNA Polymerase. The final Mg^{++} concentration in 1X Crimson 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 $MgSO_4$.

   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. Crimson LongAmp *Taq* DNA Polymerase concentration:
   We generally recommend using Crimson LongAmp *Taq* DNA Polymerase at a concentration of 100 units/ml (5 units/50 µl reaction). However, the optimal concentration of Crimson LongAmp *Taq* DNA Polymerase may vary 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)

CERTIFICATE OF ANALYSIS

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 60–65°C	10–30 seconds 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 Crimson 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 are the advantages or disadvantages of Crimson LongAmp Taq DNA Polymerase?*

   The Crimson LongAmp *Taq* Reaction Buffer formulation offers three convenient features. First, the 5X Crimson LongAmp *Taq* Reaction Buffer contains a red dye, which serves as a visual indicator of homogeneous reaction setup. Second, the 5X Crimson LongAmp *Taq* Reaction buffer facilitates direct loading of PCR products on a gel. Third, the trace amount of red dye in Crimson LongAmp *Taq* Reaction Buffer works as a tracking dye during electrophoresis.

   If the PCR products will be analyzed by absorbance or fluorescence excitaiton, acid red, (λmax = 510 nm) may interfere with the assays; Therefore LongAmp *Taq* Reaction Buffer is recommended.

2. *How do I remove the dye from my PCR reactions using Crimson LongAmp Taq DNA Polymerase?*

   Spin Colums for PCR clean-up can be used to remove the dye.

3. *What is the recommended enzyme amount when using Crimson LongAmp?*

   In general, we recommend 5 units of Crimson 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.

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

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

5. *What is the extension rate when using Crimson 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 Crimson LongAmp Taq DNA Polymerase?*

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

7. *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 Crimson LongAmp 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 *Taq* PCR Kit
#E5200S 100 Reactions

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

LongAmp *Taq* DNA Polymerase
#M0323S 500 units
#M0323L 2,500 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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