Crimson *Taq* DNA Polymerase

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M0324S 014121214121

M0324S

200 units **5,000 U/ml** **Lot: 0141212**
RECOMBINANT **Store at –20°C** **Exp: 12/14**

Description: *Taq* DNA Polymerase is a thermostable DNA polymerase that possesses a 5´→ 3´ polymerase activity (1,2,3) and a 5´ flap endonuclease activity (4,5).

Crimson *Taq* DNA Polymerase offers superior performance in its newly formulated PCR buffer. Crimson *Taq* Reaction Buffer contains a density reagent, which allows direct loading of PCR products onto a gel. In addition, Crimson *Taq* Reaction Buffer has trace amounts of a red dye, which serves as an indicator for homogenous reaction setup, a color aid in gel loading and a tracking dye which migrates at about 10 bp on a 1% TBE gel.

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1.

Applications:
- Primer Extension
- Routine PCR
- Colony PCR
- DHPLC
- Microarray Analysis
- 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 *Taq* Reaction Buffer

Reaction Conditions: 1X Crimson *Taq* Reaction Buffer, DNA template, primers, 200 µM dNTPs (not included) and 1.25–2.5 units of Crimson *Taq* DNA Polymerase in a total reaction volume of 50 µl.

1X Crimson *Taq* Reaction Buffer:
12.5 mM Tricine
42.5 mM KCl
1.5 mM $MgCl_2$
6% Dextran
Acid Red
pH 8.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 15 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.

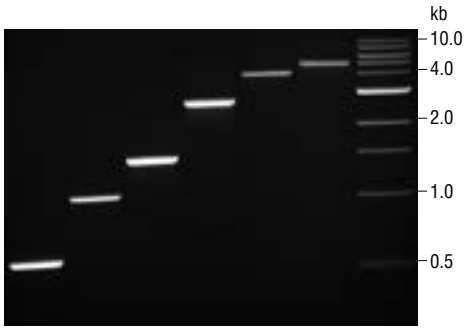
Heat Inactivation: No

Quality Control Assays

5 kb Lambda PCR: 25 cycles of PCR amplification of 5 ng Lambda DNA with 1.25 units of Crimson *Taq* DNA Polymerase in the presence of 200 µM dNTPs and 0.2 µM primers in Crimson *Taq* Reaction Buffer results in the expected 5 kb product.

3´→ 5´ Exonuclease Activity: Incubation of a 20 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of Crimson *Taq* DNA Polymerase with 10 nM fluorescent internally labeled oligonucleotide for 30 minutes at either 37°C or 75°C yields no detectable 3´→ 5´ degradation as determined by capillary electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of Crimson *Taq* DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.



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

PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (6). *Taq* DNA Polymerase is an enzyme widely used in PCR (7). The following guidelines are provided to ensure successful PCR using New England Biolabs' Crimson *Taq* DNA Polymerase. These guidelines cover routine PCR reactions. Amplification of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 5 kb 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 (95°C).

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X Crimson *Taq* Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Crimson *Taq* DNA Polymerase	0.125 µl	0.25 µl	1.25 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. 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 95°C and begin thermocycling:

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 45–68°C 68°C	15–30 seconds 15–60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4–10°C	

General Guidelines:

1. Template:
Use of high quality, purified DNA templates greatly enhances the success of PCR reactions. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	AMOUNT
Genomic	1 ng–1 µg
Plasmid or Viral	1 pg–1 ng

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. 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 *Taq* DNA Polymerase. The final Mg^{++} concentration in 1X Crimson *Taq* Reaction Buffer is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.5 or 1.0 mM increments using $MgCl_2$.
Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (8) or formamide (9).

4. Deoxynucleotides:
The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. Crimson *Taq* DNA Polymerase Concentration:
We generally recommend using Crimson *Taq* DNA Polymerase at a concentration of 25–50 units/ml (1.25–2.5 units/50 µl reaction). However, the optimal concentration of Crimson *Taq* DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications.

6. Denaturation:
An initial denaturation of 30 seconds at 95°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 95°C is recommended prior

(see other side)

CERTIFICATE OF ANALYSIS

to PCR cycling to fully denature the template . With colony PCR, an initial 5 minute denaturation at 95°C is recommended.

During thermocycling a 15–30 second denaturation at 95°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 45–68°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 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°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	95°C	30 seconds
30 Cycles	95°C 60–68°C	15–30 seconds 1 minute/kb
Final Extension	60–68°C	5 minutes
Hold	4–10°C	

11. PCR product:
The PCR products generated using Crimson *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 Taq DNA Polymerase?*

   The Crimson *Taq* Reaction Buffer formulation offers three convenient features. First, the 5X Crimson *Taq* Reaction Buffer contains a red dye, which serves as a visual indicator of homogeneous reaction setup. Second, the 5X Crimson *Taq* Reaction Buffer contains a density reagent, dextran, which allows direct loading of PCR products on a gel. Third, the trace amount of red dye in Crimson *Taq* Reaction Buffer works as a gel tracking dye (migrates at about 10 bp) during electrophoresis.

   If the PCR products will be analyzed by absorbance or fluorescence excitation, acid red (λmax= 510 nm) may interfere with the assay; therefore Standard *Taq* Reaction Buffer is recommended.

2. *Does the 5X Crimson Taq Reaction Buffer offer amplification efficiency similar to that of Standard Taq Reaction Buffer or ThermoPol Reaction Buffer?*

   Yes.

3. *Can the PCR product be used directly in T/A cloning?*

   Yes. PCR products can be directly ligated to dT/dU-overhang vectors and then transformed directly into host cells.

4. *How do I remove the dye and dextran from my PCR reactions using Crimson Taq Reaction Buffer?*

   Spin columns for PCR clean-up can be used to remove both the dye and dextran.

5. *How long is the buffer stable in non-optimal storage conditions?*

   The buffer is best stored at -20°C, but full activity is retained in buffer that is stored at 4°C for two weeks. Storage at room temperature is not recommended for more than 4 days.

References:

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4. Longley, M.J., Bennett, S.E. and Mosbaugh D.W. (1990) *Nucleic Acids Res.,* 18, 7317–7322.
5. Lyamichev, V., Brow, M.A. and Dahlberg, J.E. (1993) *Science,* 260, 778–783.
6. Saiki R.K. et al. (1985) *Science,* 230, 1350–1354.
7. Powell, L.M. et al. (1987) *Cell,* 50, 831–840.
8. Sun, Y., Hegamyer, G. and Colburn, N. (1993) *Biotechniques,* 15, 372–374.
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Companion Products Sold Separately:

Crimson *Taq* Reaction Buffer Pack
#B0324S 6.0 ml

Crimson *Taq* (Mg-free) Reaction Buffer Pack
#B0325S 6.0 ml

Diluent F
#B8006S 4.0 ml

Magnesium Chloride ($MgCl_2$) Solution
#B9021S 6.0 ml

Taq PCR Kit
#E5000S 200 Reactions

Taq 2X Master Mix
#M0270S 100 Reactions
#M0270L 500 Reactions

Quick-Load® *Taq* 2X Master Mix
#M0271S 100 Reactions
#M0271L 500 Reactions

Taq 5X Master Mix
#M0285S 100 Reactions
#M0285L 500 Reactions

Crimson *Taq* DNA Polymerase with (Mg-free) Buffer
#M0325S 200 units
#M0325L 1,000 units

Deoxynucleotide Solution Set
#N0446S 25 µmol each

Deoxynucleotide Solution Mix
#N0447S 8 µmol each
#N0447L 40 µmol each