Taq DNA Polymerase with ThermoPol™ II (Mg-free) Buffer

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400 units **5,000 U/ml** **Lot: 0141206**

RECOMBINANT **Store at –20°C** **Exp: 6/14**

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).

It is supplied with 10X ThermoPol II (Mg-free) Reaction Buffer and $MgSO_4$. 10X ThermoPol II (Mg-free) Reaction Buffer contains a nonionic detergent to increase enzyme stability during longer incubations.

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

Application:
- PCR
- Primer extension
- Colony PCR
- Long PCR (> 5 kb)

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:
10X ThermoPol II (Mg-free) Reaction Buffer
100 mM $MgSO_4$

Reaction Conditions: 1X ThermoPol II (Mg-free) Reaction Buffer, DNA template, primers, 200 µM dNTPs (not included), 2 mM $MgSO_4$ and 1.25 units of *Taq* DNA Polymerase in a total reaction volume of 50 µl.

1X ThermoPol II (Mg-free) Reaction Buffer:
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
10 mM KCl
0.1% Triton® X-100
pH 8.8 @ 25°C

Supplied with ThermoPol II (Mg-free) Reaction Buffer

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 *Taq* DNA Polymerase in the presence of 200 µM dNTPs, 0.2 µM primers and 2 mM $MgSO_4$ in ThermoPol II (Mg-free) 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 *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 *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.

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' *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
10X ThermoPol II (Mg-free) Reaction Buffer	2.5 µl	5 µl	1X
100 mM $MgSO_4$	0.5 µl	1 µl	2 mM
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)
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 *Taq* DNA Polymerase. The Mg-free buffer formulation along with supplemental $MgSO_4$ solution gives the user complete control over the final Mg^{++} concentration in the reaction.

   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. *Taq* DNA Polymerase Concentration:
   We generally recommend using *Taq* DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction). However, the optimal concentration of *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 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	15–30 seconds
	60–68°C	1 minute/kb
Final Extension	60–68°C	5 minutes
Hold	4–10°C	

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

References:

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3. Lawyer, F.C. et al. (1993) *PCR Methods and Appl.,* 2, 275–287.
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.
9. Sarkar, G., Kapelner, S. and Sommer, S.S. (1990) *Nucleic Acids Res.,* 18, 7465.

Companion Products Sold Separately:

Magnesium Sulfate ($MgSO_4$) Solution
#B1003S 6.0 ml

Diluent F
#B8006S 4.0 ml

ThermoPol Reaction Buffer Pack
#B9004S 6.0 ml

ThermoPol II (Mg-Free) Reaction Buffer Pack
#B9005S 6.0 ml

ThermoPol DF (Detergent-Free) Reaction Buffer Pack
#B9013S 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

Deoxynucleotide Solution Set
#N0446S 25 µmol each

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