

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



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M0321S 014130615061

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400 units 5,000 U/ml Lot: 0141306

RECOMBINANT Store at -20°C Exp: 6/15

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 MgSO₄. 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 MgSO₄

Reaction Conditions: 1X ThermoPol II (Mg-free) Reaction Buffer, DNA template, primers, 200 μM dNTPs (not included), 2 mM MgSO₄ 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 (NH₄)₂SO₄
10 mM KCl
0.1% Triton® X-100
pH 8.8 @ 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 [³H]-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 MgSO₄ 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)
<i>Taq</i> 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	15–30 seconds
	45–68°C	15–60 seconds
	68°C	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 MgSO₄ 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).

Supplied with ThermoPol II (Mg-free) Reaction Buffer

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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- Sarkar, G., Kapelner, S. and Sommer, S.S. (1990) *Nucleic Acids Res.*, 18, 7465.

Companion Products Sold Separately:

- Magnesium Sulfate (MgSO₄) 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

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