

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



M0321S



400 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).

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

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

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 [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 10nM 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	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).

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:

- Chien, A., Edgar, D.B. and Trela, J.M. (1976) *J. Bact.*, 127, 1550–1557.
- Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) *Biokhimiya*, 45, 644–651.
- Lawyer, F.C. et al. (1993) *PCR Methods and Appl.*, 2, 275–287.
- Longley, M.J., Bennett, S.E. and Mosbaugh D.W. (1990) *Nucleic Acids Res.*, 18, 7317–7322.
- Lyamichev, V., Brow, M.A. and Dahlberg, J.E. (1993) *Science*, 260, 778–783.
- Saiki R.K. et al. (1985) *Science*, 230, 1350–1354.
- Powell, L.M. et al. (1987) *Cell*, 50, 831–840.
- Sun, Y., Hegamyer, G. and Colburn, N. (1993) *Biotechniques*, 15, 372–374.
- 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

 Annealing temperature



NEW ENGLAND BIOLABS[®], QUICK-LOAD[®] and THERMOPOL[®] are registered trademarks of New England Biolabs, Inc.

IGEPAL[®] is a registered trademark of Rhodia Operations.

TRITON[®] is a registered trademark of Union Carbide Corporation.

TWEEN[®] is a registered trademark of Uniqema Americas LLC.