

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



1-800-632-7799  
info@neb.com  
www.neb.com



M0321S 014140616061

# M0321S



**400 units**      **5,000 U/ml**      **Lot: 0141406**  
**RECOMBINANT** Store at **-20°C** Exp: **6/16**

**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<sub>4</sub>. 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 MgSO<sub>4</sub>

**Reaction Conditions:** 1X ThermoPol II (Mg-free) Reaction Buffer, DNA template, primers, 200 μM dNTPs (not included), 2 mM MgSO<sub>4</sub> 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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
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 [<sup>3</sup>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<sub>4</sub> 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 <sub>4</sub>                   | 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<sup>++</sup> and additives:

Mg<sup>++</sup> 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<sub>4</sub> solution gives the user complete control over the final Mg<sup>++</sup> 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<sub>m</sub> 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<sub>m</sub>. We recommend using NEB's T<sub>m</sub> Calculator, available at [www.neb.com/TmCalculator](http://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:

- Chien, A., Edgar, D.B. and Trela, J.M. (1976) *J. Bact.*, 127, 1550–1557.
- Kaledin, A.S., Sliusarenko, A.G. and Goro-detskii, 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 Mos-baugh 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 (MgSO<sub>4</sub>) Solution  
#B1003S 6.0 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



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