

T4 DNA Polymerase



M0203S 040141216121

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150 units **3,000 U/ml** **Lot: 0401412**
RECOMBINANT **Store at -20°C** **Exp: 12/16**

Description: T4 DNA Polymerase catalyzes the synthesis of DNA in the 5' → 3' direction and requires the presence of template and primer. This enzyme has a 3' → 5' exonuclease activity which is much more active than that found in DNA Polymerase I. Unlike *E. coli* DNA Polymerase I, T4 DNA Polymerase does not have a 5' → 3' exonuclease function.

Source: Purified from a strain of *E. coli* that carries the T4 DNA Polymerase gene.

Applications:

- Removal of 3' overhangs to form blunt ends (1,2).
- Fill-in of 5' overhangs fill-in to form blunt ends (1,2).
- Single strand deletion subcloning (3).
- Second strand synthesis in site-directed mutagenesis (4).
- Probe labeling using replacement synthesis (1,2).

Supplied in: 100 mM KPO₄ (pH 6.5), 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2.1

Reaction Conditions: 1X NEBuffer 2.1. Supplement with dNTPs (not included).

1X NEBuffer 2.1:

50 mM NaCl
10 mM Tris-HCl
10 mM MgCl₂
100 µg/ml BSA
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C (5).

Unit Assay Conditions: 1X NEBuffer 2.1, 33 µM dNTPs including [³H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

Molecular Weight: 104,000 daltons.

Heat Inactivation: 75°C for 20 minutes.

Quality Control Assays

Endonuclease Activity: Incubation of a 50 µl reaction in NEBuffer 2.1 containing a minimum of 50 units of T4 DNA Polymerase with 1 µg of supercoiled φX174 DNA for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Protocol for blunting ends by 3' overhang removal and 3' recessed (5' overhang) end fill-in:

1. DNA should be dissolved in 1X reaction buffer* supplemented with 100 µM dNTPs.
2. Add 1 unit T4 DNA Polymerase per microgram DNA.
3. Incubate 15 minutes at 12°C.
4. Stop reaction by adding EDTA to a final concentration of 10 mM and heating to 75°C for 20 minutes (1,2).

CAUTION: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3' → 5' exonuclease activity of the enzyme.

* T4 DNA Polymerase can be used in NEBuffers 1.1, 2.1, and CutSmart Buffer as well as NEBuffers 1, 2, and 4 and T4 DNA Ligase Reaction Buffer. Optimal activity is observed in NEBuffer 2.1. BSA supplementation is recommended when using a buffer that does not already contain BSA.

(see other side)

CERTIFICATE OF ANALYSIS

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* T4 DNA Polymerase can be used in NEBuffers 1.1, 2.1, and CutSmart Buffer as well as NEBuffers 1, 2, and 4 and T4 DNA Ligase Reaction Buffer. Optimal activity is observed in NEBuffer 2.1. BSA supplementation is recommended when using a buffer that does not already contain BSA.

(see other side)

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Notes on Use:

For fill-in reactions only: T4 DNA Polymerase can be used in NEBuffers 1.1, 2.1, 3.1 and CutSmart™ Buffer as well as NEBuffers 1-4 and T4 DNA Ligase Reaction Buffer.

For blunting reactions requiring removal of overhangs: T4 DNA Polymerase can be used in NEBuffers 1.1, 2.1, and CutSmart Buffer as well as NEBuffers 1, 2, and 4 and T4 DNA Ligase Reaction Buffer. NEBuffers 3.1 and 3 are not recommended when overhang removal is required.

Optimal activity is observed in NEBuffer 2.1.

Supplement with dNTPs.*

BSA supplementation is recommended when using a buffer that does not already contain BSA.

Incubate at temperature suggested for specific protocol.

*Refer to specific protocol to determine recommended dNTP concentrations.

References:

1. Tabor, S. and Struhl, K. (1989). DNA-Dependent DNA Polymerases. In F. M. Ausebel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (Eds.), *Current Protocols in Molecular Biology* (pp. 3.5.10–3.5.12). New York: John Wiley & Sons Inc.
2. Sambrook, J. et al. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.), (pp. 5.44–5.47). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
3. Dale, R. et al. (1985) *Plasmid* 13, 31–40.
4. Kunkel, T. A. et al. (1987) *Methods Enzymology* 154, 367–382.
5. Panet, A. et al. (1973) *Biochemistry* 12, 5045–5050.

Companion Products Sold Separately:

NEBuffer 2.1
#B7202S 5.0 ml

BSA, Molecular Biology Grade
#B9000S 12 mg

Deoxynucleotide Solution Set
#N0446S 25 µmol of each

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



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