T4 DNA Polymerase

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

M0203S 040120614061

M0203S

RR NEB2 BSA Yes

150 units	**3,000 U/ml**	**Lot: 0401206**
RECOMBINANT	**Store at –20°C**	**Exp: 6/14**

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 $KP0_4$ (pH 6.5), 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2, 100X BSA

Reaction Conditions: 1X NEBuffer 2.
Supplement with 100 µg/ml BSA and dNTPs (not included).

T4 DNA Polymerase is active in all four NEBuffers and T4 DNA Ligase Reaction Buffer when supplemented with dNTPs and BSA.

Supplement with 100 µg/ml BSA and dNTPs* (not included in supplied 10X buffer). Incubate at temperature suggested for specific protocol.

*Refer to specific protocol to determine recommended dNTP concentrations.

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
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, 33 µM dNTPs including [^{3}H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

Molecular Weight: 112,000 daltons.

Heat Inactivation: 75°C for 20 minutes.

Quality Control Assays

Endonuclease Activity: Incubation of a 50 µl reaction in NEBuffer 2 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.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	60%
NEBuffer 2	**100%**
NEBuffer 3	100%
NEBuffer 4	100%

Notes on Use: Protocol for blunting ends by 3´ overhang removal and 3´ recessed end fill-in: DNA should be dissolved in 1X NEBuffer 1–4 or T4 DNA Ligase Reaction Buffer supplemented with 100 µM dNTPs. Add 1 unit T4 DNA Polymerase per microgram DNA and incubate 15 minutes at 12°C. 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.

(see other side)

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(see other side)

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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
#B7002S 6.0 ml

Bovine Serum Albumin (BSA)
#B9001S 6.0 ml

Deoxynucleotide Solution Set
#N0446S 25 µmol of each

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

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.
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