T7 DNA Polymerase (unmodified)

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

M0274S 00913031 5031

M0274S

RR BSA Yes

300 units **10,000 U/ml** **Lot: 0091303**

RECOMBINANT **Store at –20°C** **Exp: 3/15**

Description: T7 DNA Polymerase catalyzes the replication of T7 phage DNA during infection. The protein dimer has two catalytic activities: DNA polymerase activity and strong 3´→ 5´ exonuclease (1,2,3). The high fidelity and rapid extension rate of the enzyme make it particularly useful in copying long stretches of DNA template.

Source: T7 DNA Polymerase consists of two subunits: T7 gene 5 protein (84 kilodaltons) and *E. coli* thioredoxin (12 kilodaltons) (1,4–7). Each protein is cloned and overexpressed in a T7 expression system in *E. coli* (4).

Applications:

- Second strand synthesis in site-directed mutagenesis protocols (8).

Supplied in: 50 mM $KP0_4$ (pH 7.0), 0.1 mM EDTA, 1 mM dithiothreitol, and 50% glycerol.

Reagents Supplied with Enzyme:
10X T7 DNA Polymerase Reaction Buffer, 10 mg/ml BSA.

Reaction Conditions: 1X T7 DNA Polymerase Reaction Buffer supplemented with 50 µg/ml BSA and dNTPs (not included), DNA and T7 DNA Polymerase incubated at 37˚C

1X T7 DNA Polymerase Reaction Buffer:
20 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.5 @ 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.

Unit Assay Conditions: 100 mM KCl, 20 mM Tris-HCl (pH 7.6), 6 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM EDTA, 50 µg/ml BSA, 150 µM dNTPs including [^{3}H]-dTTP and 0.162 mg/ml activated calf thymus DNA.

Heat Inactivation: 75°C for 20 minutes.

Quality Control Assays

Endonuclease Activity: Incubation of a 50 µl reaction in T7 DNA Polymerase Reaction Buffer containing a minimum of 100 units of T7 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.

Notes On Use: The high polymerization rate of the enzyme makes long incubations unnecessary.

T7 DNA Polymerase is not suitable for DNA sequencing.

References:

1. Hori, K. et al. (1979) *J. Biol. Chem.* 254, 11598–11604.
2. Engler, M. J. et al. (1983) *J. Biol. Chem.* 258, 11165–11173.
3. Nordstrom, B. et al. (1981) *J. Biol. Chem.* 256, 3112–3117.
4. Studier, F. W. et al. (1990) *Methods Enzymol.* 185, 60–89.
5. Grippo, P. and Richardson, C. C. (1971) *J. Biol. Chem.* 246, 6867–6873.
6. Modrich, P. and Richardson, C. C. (1975) *J. Biol. Chem.* 250, 5515–5522.
7. Adler, S. and Modrich P. (1979) *J. Biol. Chem.* 254, 11605–11614.
8. Bebenek, K. and Kunkel, T. A. (1989) *Nucleic Acids Res.* 17, 5408.

Companion Products Sold Separately:

BSA	
#B9001S	6.0 ml
Deoxynucleotide Solution Set	
#N0446S	25 µmol each
Deoxynucleotide Solution Mix	
#N0447S	8 µmol each
#N0447L	40 µmol each

CERTIFICATE OF ANALYSIS

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BSA
#B9001S 6.0 ml
Deoxynucleotide Solution Set
#N0446S 25 µmol each
Deoxynucleotide Solution Mix
#N0447S 8 µmol each
#N0447L 40 µmol each

CERTIFICATE OF ANALYSIS