T7 DNA Polymerase (unmodified)

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

Supplied in: 50 mM KPO4 (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 MgCl2
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), 0.5 mM DTT, 0.1 mM EDTA, 50 μg/ml BSA, 150 μM dNTPs including [3H]-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 pX174 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.

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Companion Products Sold Separately:
BSA
#89001S 6.0 ml
Deoxynucleotide Solution Set
#N0446S 25 μmol each
Deoxynucleotide Solution Mix
#N0447S 8 μmol each
#N0447L 40 μmol each

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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).

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