T4 DNA Polymerase

**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, 100X BSA

**Reaction Conditions:**
1X NEBuffer 2:
- 50 mM NaCl
- 10 mM Tris-HCl
- 10 mM MgCl2
- 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 [3H]-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.

**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.

**Enzyme Properties**

**Activity in NEBuffers:**
- NEBuffer 1: 100%
- NEBuffer 2: 100%
- NEBuffer 3: 100%
- NEBuffer 4: 100%

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References:

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