



## T4 DNA Polymerase

Catalog Number: E105-1, E105-2

Table 1. Kit Components and Storage

Kit Component	E105-1 (150 units)	E105-2 (750 units)	Storage	Stability
T4 DNA Polymerase (3 units/ $\mu$ L)	50 $\mu$ L	250 $\mu$ L	-20 °C, avoid repeated free-thaw	The product is stable for at least 6 months when stored as directed.
10x Reaction Buffer	250 $\mu$ L	1.25 mL		

### Product Description

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

The enzyme is available in 150 and 750 unit sizes at a concentration of 3 U/ $\mu$ L. The enzyme is supplied with a 10x Reaction Buffer.

### Applications

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

### Product Specifications

- **Storage Buffer:** 100 mM KPO<sub>4</sub> (pH 6.5 at 25 °C), 1 mM DTT, and 50% (v/v) glycerol.
- **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:** 1X reaction buffer, 33  $\mu$ M dNTPs including [<sup>3</sup>H]-dTTP, 70  $\mu$ g/ml denatured herring sperm DNA.

### Protocol for blunting ends by 3' overhang removal and 5' overhang end fill-in

1. Dissolve DNA sample in 1X reaction buffer supplemented with 100  $\mu$ M dNTPs.
2. Add 1 unit T4 DNA Polymerase per microgram DNA.
3. Incubate at 12 °C for 15 min.
4. Stop the reaction by heating at 75 °C for 20 min or adding EDTA to a final concentration of 10 mM.