

## T4 DNA Ligase

### *E. coli* lambda lysogen NM 989

	Cat.-No.	Size	Conc.
	EN-149S	15,000 units	150 units/ $\mu$ l
	EN-149L	75,000 units	150 units/ $\mu$ l

For *in vitro* use only  
Quality guaranteed for 12 months  
Store at -20°C

#### Avoid freeze / thaw cycles

#### T4 DNA Ligase

150 units/ $\mu$ l T4 DNA Ligase in 10 mM Tris-HCl (pH 7.4), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200  $\mu$ g/ml BSA and 50% glycerol.

#### 10x Reaction Buffer

50 mM Tris-HCl (pH 7.8), 10 mM MgCl<sub>2</sub>, 10 mM DTT and 1 mM ATP.

#### Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA.

#### Reaction conditions

50 mM Tris-HCl (pH 7.8), 10 mM MgCl<sub>2</sub>, 10 mM DTT, 1 mM ATP and DNA (0.1 to 1  $\mu$ M in 5'-termini). Optimal ligation occurs at 16°C.

#### Unit definition

One Weiss unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of  $\lambda$  DNA (5' DNA termini concentration of 0.12  $\mu$ M, 300  $\mu$ g/ml) in a total reaction volume of 20  $\mu$ l in 30 minutes at 16°C in 1x T4 DNA Ligase reaction Buffer.

#### Heat inactivation

T4 DNA Ligase can be inactivated by incubation at 65°C for 10 minutes.

#### Notes

- One unit is equivalent to 0.015 Weiss units.
- One Weiss unit is defined as the amount of enzyme required to catalyze the exchange of 1 nmol of <sup>32</sup>P from pyrophosphate to ATP, into Norit-adsorbable material in 20 minutes at 37°C.
- T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 200 mM.
- Ligation of blunt-ended and single-base pair overhang fragments requires about 50 times as much enzyme to achieve the same extent of ligation as cohesive-end DNA fragments. Blunt-end ligation may be enhanced by addition of PEG 4000 (10% w/v final concentration) or hexamine chloride, or by reducing the ATP concentration to 50  $\mu$ M.
- To dilute T4 DNA Ligase for subsequent storage at -20°C a storage buffer containing 50% glycerol should be used; to dilute Ligase for immediate use, 1x Reaction Buffer is recommended.