

Data Sheet

# T4 DNA Ligase

Source: *E. coli lambda lysogen NM 989*

Cat.-No.	Amount	Conc.
mi-E0149S	15 000 Units	150 u/µl
mi-E0149L	75 000 Units	150 u/µl

**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 µM.
- To dilute T4 DNA Ligase that will subsequently be stored at -20°C, 50% glycerol storage buffer should be used; to dilute for immediate use, 1x T4 DNA Ligase reaction buffer can be used.

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

**Unit definition:** One unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of λ DNA (5' DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16°C in 1x T4 DNA Ligase reaction Buffer.

**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 µM in 5'-termini). Optimal ligation occurs at 16°C.

**Storage buffer:** 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol. Store at -20°C.

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