



T4 DNA Ligase

E. coli lambda lysogen NM 989

Cat. No.	Amount
EN-149S	400 Weiss units
EN-149L	5 x 400 Weiss units

Unit Definition: One Weiss unit is defined as the amount of enzyme required to catalyze the exchange of 1 nmol of ^{32}P from pyrophosphate to ATP, into Norit-adsorbable material in 20 minutes at 37 °C.

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid (Supplied in 10 mM Tris-HCl pH 7.4, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50 % [v/v] glycerol)

Concentration: 2.5 WU/µl

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:

For a typical ligation of 100 - 200 ng vector DNA 0.1 - 1 Weiss Units of T4 DNA Ligase are used. Optimal ligation occurs at 16 °C.

10x Reaction Buffer:

500 mM Tris-HCl pH 7.8 at 25 °C, 100 mM MgCl_2 , 100 mM DTT, 10 mM ATP and 25 µg/ml BSA.

Heat inactivation:

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

Note:

- One Cohesive-End Ligation Unit (CEU) 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.
- One Weiss unit is equivalent to approx. 67 CEU.
- 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 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.