

**Description:**

T4 DNA Ligase catalyzes the formation of a phosphodiester bonds between 5' phosphate and 3' hydroxyl termini in duplex DNA/RNA. This enzyme can join blunt end and cohesive end termini, repair single stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids.

**Applications:**

- Cloning of restriction fragments
- joining linkers and adapters to blunt-ended DNA
- gene (gene fragments) synthesis.

**Concentration:** 2,5 Weiss Units /  $\mu$ l

**Source:**

Isolated from E.coli strain that carries the cloned DNA ligase gene from bacteriophage T4

**Usage:**

For most cohesive end ligations, a 30 minute incubation at 20°C is sufficient. Incubations at 16°C for 4-16 hours are routinely used for the majority of applications.

Ligation of blunt ends and single-base pair overhang fragments requires more enzyme to achieve the same extent of ligation as cohesive end DNA fragments. Ligation may be enhanced by addition of PEG, or by reducing the rATP concentration. ATP is an essential cofactor for the reaction.

**Storage buffer:**

10 mM Tris-HCl pH 7.4, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200  $\mu$ g/ml BSA and 50 % [v/v] glycerol)

**Unit definition:**

One unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of lambda DNA in 30 minutes at 16°C at 5' termini concentration of 0.12  $\mu$ M (300  $\mu$ g/ml). One Cohesive End Ligation Unit equals 0.015 Weiss units. One Weiss unit equals 67 Cohesive End Ligation Units.

**Reaction buffer (10X):**

500 mM Tris-HCl pH 7.8 at 25 °C, 100 mM MgCl<sub>2</sub>, 100 mM DTT, 10 mM ATP and 25  $\mu$ g/ml BSA.

**Quality Assurance:** Free of contaminating exonuclease and endonuclease

**Storage:** shipped on blue ice, 24 months at -20°C

## Notes:

- One Cohesive-End Ligation Unit (CEU) 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.
- 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  $\mu$ 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.

## Order Information

Prod. No.	Description	Quantity
4902-002	T4 DNA ligase	400 WEISS UNITS
4902-010	T4 DNA ligase	2000 WEISS UNITS
4902-015	T4 DNA ligase	6000 WEISS UNITS