Recombinant T4 DNA Ligase

Catalog No.	CSI12682	**Quantity:**	2 X 10E4 U
	CSI12683		1 X 10E5 U
	CSI12684		5 X 10E5 U

Alternate Names: DNA ligase 4, EC 6.5.1.1, DNA ligase IV, Polydeoxyribonucleotide synthase [ATP] 4.

Description: T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' -phosphate and 3' -hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

Physical Appearance: Sterile filtered liquid formulation having a concentration of 167,000 U/ml.

Source: *E. coli* lambda lysogen NM 989.

Formulation: 50 mM Tris-HCl (pH 7.8 at 25°C + 10 mM $MgCl_2$ + 10 mM DTT + 1 mM ATP + 25 µg/ml BSA and DNA (0.1 to 1 µm in 5´ termini). Optimal ligation occurs at 16°C.

Nuclease Activity: Incubation of 13,000 units for 18 hours in assay buffer (without ATP) with Hind III fragments of gamma DNA yielded a clear and sharp banding pattern on agarose gels.

Endonuclease Activity: Incubation of a 50 µl reaction containing 13,000 units of T4 DNA Ligase with 1 µg of X 174 RF I DNA for 4 hours at 37°C resulted in < 5% conversion to RFII as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 13,000 units of T4 DNA Ligase with 1 µg of a mixture of single and double-stranded [3H] *E. coli* DNA (200,000 cpm/ug) for 4 hours at 37°C released < 0.3% of the total radioactivity.

Biological Activity: One Weiss unit is equivalent to circa 67 cohesive-end ligation units.
T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration is > 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, 1 x T4 DNA Ligase reaction buffer can be used.

Quality Control: Purified free of contaminating endonucleases and exonucleases. Each lot of T4 DNA ligase is also tested in a mock cloning assay, which reveals any damage to the ligated DNA termini. Greater than 99.9% of the termini remain undamaged in this assay.

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

Unit Definition: 1. One unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of DNA (5´ DNA termini concentration of 0.12 µM, 300- µg/ml) in a total reaction volume of 20 ul in 30 minutes at 16°C in 1 X T4 DNA Ligase Reaction Buffer. 2. One Weiss unit is defined as the amount of enzyme required to catalyze the exchange of 1 nmol of 32P from pyrophosphate to ATP, into Norit-adsorbable material in 20 minutes at 37°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.

Storage & Stability: Two years when stored at -20°C, 2 weeks at 4°C.

Applications: Cloning of restriction fragments.
Joining linkers and adapters to blunt-ended DNA.

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