# T4 DNA Ligase

1-800-632-7799
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M0202S 102120314031

# M0202S

RR Yes!

**20,000 units 400,000 cohesive end units/ml Exp: 3/14**

**RECOMBINANT Store at –20°C Lot: 1021203**

**Description:** 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 (1).

**Source:** Purified from *E. coli* C600 pcl857 pPLc28 lig8 (2)

**Applications:**
- Cloning of restriction fragments (3)
- Joining linkers and adapters to blunt-ended DNA

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

**Reagents Supplied with Enzyme:**
10X T4 DNA Ligase Reaction Buffer.

**Reaction Conditions:** 1X T4 DNA Ligase Reaction Buffer. Incubate at 16°C.

**1X T4 DNA Ligase Reaction Buffer:**
50 mM Tris-HCl
10 mM $MgCl_2$
10 mM DTT
1 mM ATP
pH 7.5 @ 25°C

Recommended DNA concentration (0.1 to 1 µM of 5´ termini).

**Unit Definition:** (Cohesive End Ligation Unit): One NEB unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (5´ DNA termini concentration of 0.12 µM [300 µg/ml]) in 20 µl of 1X T4 DNA Ligase Reaction Buffer in 30 minutes at 16°C.

**Heat Inactivation:** 65°C for 10 minutes.

## Quality Control Assays

**Exonuclease Activity:** Incubation of 3,200 units of enzyme with 1 µg sonicated $^3H$ DNA (2 x $10^5$ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

**Nuclease Activity:** Incubation of 3,200 units for 18 hours in assay buffer with HindIII fragments of λ DNA yielded a clear and sharp banding pattern on agarose gels.

**Endonuclease Activity:** Incubation of 3,200 units for 4 hours at 37°C in 50 µl of reaction buffer resulted in < 5% conversion to RF II.

**Room Temperature Ligation:**
For convenience, ligations may be done at room temperature (20–25°C). For cohesive (sticky) ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 10 minutes. For blunt ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 2 hours or 1 µl high concentration T4 DNA Ligase for 10 minutes.

Alternatively, NEB's Quick Ligation Kit (NEB #M2200S, [30 reactions] or NEB #M2200L, [150 reactions]) is uniquely formulated to ligate both blunt and cohesive (sticky) ends in 5 minutes at room temperature.

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

**Notes on Use**: ATP is an essential cofactor for the reaction. This contrasts with *E. coli* DNA Ligase which requires NAD.

To dilute T4 DNA Ligase that will subsequently be stored at –20°C, 50% glycerol storage buffer (Diluent Buffer A, NEB #B8001S) should be used; to dilute for immediate use, 1X T4 DNA Ligase Reaction Buffer can be used.

Ligation can also be performed in any of the four restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer if they are supplemented with 1 mM ATP.

**References:**

1. Engler, M. J. and Richardson, C. C. (1982). In P. D. Boyer (Ed.), *The Enzymes* Vol. 5, (p. 3). San Diego: Academic Press.
2. Remaut, E., Tsao, H. and Fiers, W. (1983) *Gene* 22, 103–113.
3. Sambrook, J., et al. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), (pp. 1.53–1.73) Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

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