T4 DNA Ligase

**M0202S**

20,000 units 400,000 cohesive end units/ml Exp: 8/18 RECOMBINANT Store at –20°C Lot: 1181608

**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

**Quality Control Assays**

**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.

**16-hour Non-specific Nuclease Activity Assay:** A 50 µl reaction containing 1 µg of Lambda-HindIII DNA and a minimum of 2,000 units of T4 DNA Ligase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity Assay (radioactivity release):** Incubation of a 50 µl reaction containing a minimum of 2,000 units of T4 DNA Ligase with 1 µg of a mixture of single and double-stranded [3H] *E. coli* DNA for 4 hours at 37°C releases < 0.1 % of the total radioactivity.

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

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

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References:

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