**T4 DNA Ligase**

**M0202S**

20,000 units 400,000 cohesive end units/ml Exp: 8/15
RECOMBINANT Store at –20°C Lot: 1081308

**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 pLc28 lig8 (2)

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

**Quality Control Assays**

**Exonuclease Activity:** Incubation of 3,200 units of enzyme with 1 µg sonicated 3H DNA (2 x 10^6 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 16 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 Poly-nucleotide Kinase Buffer if they are supplemented with 1 mM ATP.

**References:**

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