T4 DNA Ligase

**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.
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**Reaction Conditions:**
- **1X T4 DNA Ligase Reaction Buffer:** Incubate at 16°C.
- **10X T4 DNA Ligase Reaction Buffer:**
  - 50 mM Tris-HCl
  - 10 mM MgCl2
  - 10 mM DTT
  - 1 mM ATP
  - pH 7.5 @ 25°C
  - Recommended DNA concentration (0.1 to 1 µM of 5’ termini).

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

**Quality Control Assays**

**Exonuclease Activity:** Incubation of 3,200 units of enzyme with 1 µg sonicated λ 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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**References**