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.

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

1X 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).

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 mM [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 10⁶ cpmp/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: