T4 DNA Ligase

1-800-632-7799
info@neb.com
www.neb.com

M0202S 115150317031

M0202S

20,000 units **400,000 cohesive end units/ml** **Exp: 3/17**
RECOMBINANT **Store at –20°C** **Lot: 1151503**

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 pcI857 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

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 [^{3}H] *E. coli* DNA for 4 hours at 37°C releases < 0.1 % of the total radioactivity.

Endonuclease (nicking) Activity Assay: Incubation of a 50 µl reaction containing a minimum of 2,000 units of T4 DNA Ligase with 1 µg of PhiX174 RF I DNA for 4 hours at 37°C results in < 10 % conversion to RF II as determined by agarose gel electrophoresis.

Protein Purity by SDS PAGE: The purity of T4 DNA Ligase is a minimum of 95% 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 #B8001) 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.

(see other side)

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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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