T4 DNA Ligase

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.

Source: Purified from E. coli C600 pcl857 pPLc28 lig8 (2)

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

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 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 [3H] 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 Poly-nucleotide Kinase Buffer if they are supplemented with 1 mM ATP.

(see other side)
References: