EPICENTRE®
Biotechnologies

T4 DNA Ligase, Cloned

Cat. Nos. **L0805H**, **L0810H**, **L0820H**, **LH805H**, **LH810H**, and **LH820H**

T4 DNA Ligase is the most versatile and commonly used ligase for DNA cloning. This ATP-dependent enzyme covalently joins blunt or compatible cohesive ends, as well as nicks in double-stranded DNA. A 5'-phosphoryl group is required for ligation to a 3'-hydroxyl. T4 DNA Ligase is available in 500, 1,000 and 2,000 Unit sizes at both regular (2 U/µl) and high (10 U/µl) concentrations. High-concentration T4 DNA Ligase is useful for obtaining maximum efficiency in blunt-end ligations. Both a 10X Reaction Buffer and 25 mM ATP Solution are also provided with the enzyme.

For those interested in the shortest possible reaction times, EPICENTRE also offers Fast-Link™ DNA Ligation Kits. These kits are specially formulated to provide the fastest high efficiency DNA ligations for routine and high throughput DNA cloning. Cohesive end ligations can be performed in as little as 5 min. and blunt end ligations in as little as 15 min.

Product Specifications

Storage: Store only at -20°C in a freezer without a defrost cycle.

Storage Buffer: T4 DNA Ligase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT) and 0.1% Triton® X-100.

Unit Definition: One unit converts 1 nmole of pyrophosphate into Norit-adsorbable material in 20 minutes at 37°C.[1]

Quality Control: T4 DNA Ligase is function-tested in a 50 µl reaction containing 33 mM Tris-acetate (pH 7.8), 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT, 1 mM ATP, 1 µg *Hin*d III-cut lambda DNA and varying amounts of enzyme.

10X Reaction Buffer: is 330 mM Tris-acetate (pH 7.8), 660 mM potassium acetate, 100 mM magnesium acetate and 5 mM DTT.

ATP is not included in the 10X Reaction Buffer and must be added separately. For most applications, ATP should be added to the reaction to a final concentration of 0.5-1.0 mM.

Contaminating Activity Assays: Cloned T4 DNA Ligase is free of detectable exo- and endonuclease and RNase activities, as judged by gel electrophoresis following incubation of greater than 150 units of enzyme for 16 hours at 37°C with the following substrates: 1) a 40-mer oligodeoxynucleotide with a 5'-phosphorylated end; 2) a 40-mer oligodeoxynucleotide with a 5'-hydroxyl end; 3) *Hin*d III-digested bacteriophage lambda DNA; and 4) supercoiled pUC-19 DNA.

Note on High Concentration T4 DNA Ligase: At high concentrations, sufficient amounts of enzyme can bind to DNA causing the DNA to migrate abnormally in an agarose gel causing the DNA band to appear smeary. This phenomenon should not be mistaken as nuclease contamination as the DNA is recovered intact if the Ligase is removed from the reaction. Excess T4 DNA Ligase can also decrease transformation efficiencies.[2] Excess enzyme may be removed by adding 0.1% SDS and Proteinase K (20 µg/ml) followed by incubation at 37°C for 30 min. The reaction is then heated to 70°C for 10 min, after which the DNA can be used in standard transformation protocols.

Related Products: The following products are also available:
- Fast-Link™ DNA Ligation Kit
- Fast-Link™ DNA Ligation and Screening Kit
- Colony Fast-Screen™ Kits
- Ampligase® Thermostable DNA Ligase
- T4 Polynucleotide Kinase
- T4 RNA Ligase
- T4 DNA Polymerase
- Mung Bean Nuclease
- End-It™ DNA End-Repair Kit

References:

1. Weiss, B. *et al.*, (1968) *J. Biol. Chem.* **243**, 4543.
2. Michelsen, B.K. (1995) *Anal. Biochem.* **225**, 172.

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