T4 DNA ligase

T4 DNA ligase (3U/ µl)	100 U
10x T4 DNA Ligase buffer	200 µl

For research use only
Cat No: YT9075
Size: 100 U
Store at –20°C

Description:

YTA-T4 DNA Ligase catalyzes the joining of two strands of DNA between the 5' phosphate group of the donor and the 3' hydroxyl group of the ligand to form a 3' → 5' phosphodiester bond in either a cohesive-ended or blunt-ended configuration. the substrate of this enzyme is double stranded DNA and will not join single stranded nucleic acids.

Unit Definition: 0.01 Weiss unit of T4 DNA Ligase is defined as the amount of enzyme required to catalyze the ligation of greater than 95% of the Hind III fragments of 1µg of Lambda DNA at 16°C in 20 minutes. See the unit concentration on the Product Information Label. **Molecular Weight:** 68kDa .

Ligase Buffer, 10X : The Ligase 10X Buffer supplied with this enzyme has a composition of Tris-HCl (pH 7.8), MgCl2, DTT and ATP. The performance of this buffer depends on the integrity of the ATP. Store the buffer in small aliquots at –20°C to minimize degradation of the ATP and DTT.

Note: The DTT in the Ligase 10X Buffer may precipitate upon freezing. If this occurs, vortex the buffer until the precipitate is in solution (typically 1–2 minutes). The performance of the product is not affected provided that the precipitate is resuspended.

Enzyme Storage Buffer: T4 DNA Ligase is supplied in 10mM Tris-HCl (pH 7.4), 50mM KCl, 1mM DTT, 0.1mM EDTA and 50% glycerol.

Source: E. coli strain expressing a recombinant clone.
Storage Temperature: Store at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.
Standard Applications : Ligation of DNA
Material to Be Supplied by the User : Nuclease-Free Water

Protocol:

We recommend using a 2-6:1 molar ratio of vector : insert DNA when cloning a fragment into a plasmid vector. These ratios will vary with other types of vectors, for example, cDNA and genomic cloning vectors.

$$\frac{\text{ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}} = \text{ng of insert}$$

prepare the following reaction mixture:

Vector DNA	100ng
Insert DNA	2-6:1 17ng
10x ligase buffer	1µl
T4DNA ligase	1µl
water	Up to 10µl
Total volume	10µl

2. Incubate the reaction at room temperature for 3 hours, or 4°C overnight, or 15°C for 4–18 hours.

Example:

How much 0.5kb insert DNA should be added to a ligation in which 100ng of 3kb vector will be used? The desired vector:insert ratio will be 1:3.

$$\frac{100\text{ng vector} \times 0.5\text{kb insert}}{3\text{kb vector}} \times \frac{3}{1} = 50\text{ng insert}$$

The following ligation reaction of a 3kb vector and a 0.5kb insert DNA uses a 1:1 vector:insert ratio. Typical ligation reactions use 100–200ng of vector DNA.

Notes:

1. There is considerable latitude in the temperature and time needed for successful ligations. The optimal temperature for a ligation is a balance between the optimal temperature for T4 DNA Ligase enzyme activity (25°C) and the temperature necessary to ensure annealing of the fragment ends, which can vary with the length and base composition of the overhangs. Shorter duplexes (linkers less than 16 bases long) require lower temperatures as a result of their lower melting temperatures . In general, ligation reactions performed at lower temperatures require longer incubation times. The scientific literature reflects this variability in ligation conditions. Blunt-end ligations generally are efficient at temperatures between 15–20°C for 4–18 hours, while sticky ends are ligated effectively at room temperature (22°C) for 3 hours or 4–8°C overnight.

2. The ligation conditions given in this protocol are based on the conditions used at Promega for quality control of lambda vectors with sticky ends. These ligation conditions have been developed using Promega Blue/White Cloning-Qualified T4 DNA Ligase.

3. The addition of polyethylene glycol (PEG) to ligation reactions can promote ligation of blunt-ended fragments by “macromolecular crowding” . We do not recommend the use of PEG in ligations, however, due to extreme variability in the quality of PEG. In addition, the use of PEG can lead to undesirable concatemerization when cloning cDNAs, and residual PEG is inhibitory to lambda packaging reactions.

Additional Information

Requirements: Mg^{2+}, ATP and DTT (3). The optimum concentration of Mg^{2+} is 10mM. Mn^{2+} may be substituted for Mg^{2+} but is only 25% as effective as Mg^{2+} .

Inhibition: 50% inhibition by greater than 150mM NaCl (activity measured at nicks) Other inhibitors include 0.2M K^+, Cs^+, Li^+, NH_4^+ and 1mM spermine.

Inactivation: Heat to 70°C for 10 minutes .