

Thermus filiformis(*Tfi*) DNA Ligase

[Cat. No.]

E- 3111 (2,000 Units)

E- 3112 (10,000 Units)

[Lot No.]

[Concentration]

20units/uL

Exp. date:

● **Description** : *Tfi* DNA Ligase catalyzes the formation of a phosphodiester bonds between adjacent 3'-hydroxyl and 5'-phosphate termini in nicked duplex DNA molecules or between oligonucleotides which are in duplex with a complementary strand at 45 - 65 °C. *Tfi* DNA Ligase is stable and active at much higher temperatures than conventional DNA ligases. Thus, *Tfi* DNA Ligase is the ideal enzyme for applications the require high-temperature, high-stringency ligation of duplex DNA molecules.

● **Source** : *Tfi* DNA Ligase is isolated from *E. coli* cells containing the ligase gene cloned from *Thermus filiformis*¹.

● **Applications** : Ligase Chain Reaction(LCR)², Oligonucleotide Ligation Assay (OLA)³, Mutagenesis by Incorporation of a Phosphorylated Oligo During PCR Amplification⁴, Simultaneous Mutagenesis of Multiple Sites⁵

● Supplied with Enzyme

- 10X *Tfi* DNA Ligase Reaction Buffer (1 mL) : 300 mM Tris-HCl, 250 mM KCl, 50 mM MgCl₂, 5 mM NAD (pH 8.3)
- 1X Dilution Buffer for Enzyme(1 mL) : 10 mM Tris-HCl, 0.1 mM EDTA, 50 mM KCl, 1mM DTT, 200 ug/mL Acetylated BSA, 50 % Glycerol (pH 7.6)

● **Storage conditions** : 20 mM Tris-HCl, 2 mM MgCl₂, 1 mM EDTA, 1 mM DTT, 0.5 % Tween-20, 0.5 % IGEPAL CA-630, 50 % Glycerol (pH 7.6), store at -20 °C

● **Unit Definition** : One unit of *Tfi* DNA Ligase is defined as the amount of enzyme required to give 50 % ligation of the 12-base pair cohesive ends of 1 ug of *Psp* EI digested lambda DNA in 10 minutes at 45 °C.

● **Activity Assay Conditions** : The activity assay is carried out in a 20 uL reaction containing 1 ug of *Psp* EI digested lambda DNA and 1X *Tfi* DNA Ligase Reaction Buffer. After incubation at 45 °C for 10minutes, the reaction is terminated by addition of stop solution(40 %(w/v) sucrose, 50 mM EDTA and 0.25 % bromophenol blue). Then heat at 70 °C for 10 minutes and immediately load on a 0.8 % agarose gel.

● **Stability** : The half-life of the enzyme in 1X Reaction Buffer is more than 1 hours at 95 °C and 55 hours at 65 °C.

● **Note** : *Tfi* DNA Ligase should not be used as a substitute for other DNA ligases, i.e., T4 DNA Ligase.

● References

1. Kim HK, Kwon ST. (1998) *Mol. Cells*. **8**:4, 438-443
2. Barany, F. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 189-193.
3. Landegren, U. *et al.*(1988) *Science* **241**, 1077-1080
4. Michael, Scott F. (1994) *Biotechniques* **16**:3, 410-412.
5. Gerard J. A. *et al.* (1993) *Biotechniques* **15**:1, 172-178.

Note

For research use only. Not for use in diagnostic or therapeutic procedures.