Tfi DNA Ligase

[Cat. No.] E-3111, E-3112

Introduction

Thermus filiformis (*Tfi*) DNA ligase catalyzes the formation of phosphodiester bonds between adjacent 3'-hydroxyl and 5'-phosphate termini of nicked duplex DNA or between oligonucleotides which are in duplex with a complementary strand. As reaction is performed at higher temperatures (45-65°C) than conventional DNA ligase, it is more stable and active at higher temperatures. This product can be applied for reactions that require high temperatures, or high stringency ligation of duplex DNA.

Applications

- Ligase chain reaction (LCR)
- Oligonucleotide Ligation Assay (OLA)
- Mutagenesis by incorporation of a phosphorylated oligo during PCR amplification
- Simultaneous mutagenesis of multiple sites

Components

Components	E-3111	E-3112
Tfi DNA Ligase (20 U/µl)	2,000 U (100 µl)	10,000 U (100 µl x 5)
10X Reaction buffer	1 ml	1 ml x 5
Dilution buffer	1 ml	1 ml x 5

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

Buffer Composition

10X Reaction buffer	Contains 300 mM Tris-HCl, 250 mM KCl, 50 mM $MgCl_2$, 5 mM NAD, pH 8.3

Storage Buffer

Tfi DNA Ligase is supplied in 50% (v/v) glycerol containing 20 mM Tris-HCl, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, and stabilizers, pH 7.6.

Unit Definition

One unit is defined as the amount of enzyme required to ligate 50% of the 12-base pair cohesive ends of 1 µg of *PspE* I digested lambda DNA for 10 min at 45°C in total volume of 20 µl.

Quality Control

- Activity Assay Conditions: The activity assay is carried out in a 20 µl reaction containing 1 µg of *PspE* I digested Lambda DNA and 1X *Tfi* DNA Ligase Reaction buffer. After incubation at 45°C for 10 min, 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 min and immediately load on a 0.8% agarose gel.
- Nuclease Contamination Assay: Nuclease activity is not detected after incubation of 1 µg of DNA with 20 U of *Tfi* DNA ligase at 37°C for 16 hrs.

Stability

The half-life of the enzyme in 1X Reaction buffer is more than 1 hr at 95°C and 55 hrs at 65°C.

Enzyme Inactivation

Tfi DNA Ligase is inactivated by heating at 70°C for 10 min.

Storage

Store at -20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

Precautions

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

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols.

Ordering Information

Description		Cat. No
Tfi DNA Ligase	2,000 U (100 rxn)	E-3111
	10,000 U (500 rxn)	E-3112

Notice

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Explanation of Symbols

Experimental Procedures

Steps		Procedure Details
1	 **Thaw reagents**	1. Thaw 10X Reaction buffer and mix thoroughly before use. Then, briefly spin down all components including insert DNA, vector DNA, *Tfi* DNA Ligase, and nuclease-free water.
2	 **Preparation of reaction mixture**	2. Add insert DNA, vector DNA, 10X Reaction buffer, *Tfi* DNA Ligase, and nuclease-free water into 0.2 ml PCR tubes (not provided) to a total volume of 20 µl. • Amount of insert DNA and vector DNA $\text{Insert DNA (ng)} = \frac{\text{vector DNA (ng)} \times \text{size of insert DNA (kb)}}{\text{size of vector DNA (kb)}} \times \text{molar ratio of} \frac{\text{insert DNA}}{\text{vector DNA}}$ • Preparation of reaction mixture **Components** / **20 µl reaction** Insert DNA: Variable Vector DNA: Variable 10X Reaction buffer: 2 µl *Tfi* DNA Ligase (20 U/µl): 0.5-1 µl Nuclease-free water: Variable Total volume: 20 µl * **Note**: We recommend the molar ratio of insert DNA : vector DNA = 3 : 1. 3. Mix the reaction mixture by tapping and briefly spin down.
3	 **Incubate reaction mixture**	4. Incubate the reaction mixture at 45-65°C for 10 min. 5. Collect 10 µl from tube and perform transformation with 100 µl of competent cells. * **Note:** In case of electroporation, salts should be precipitated from ligation mixture.