T4 DNA Ligase

[Cat. No.] E-3061, E-3062

Introduction

T4 DNA ligase catalyzes the formation of phosphodiester bonds in adjacent nucleotides between the 5'-phosphate terminal and the 3'-hydroxyl terminal of duplex DNA or RNA. This product can repair not only single-strand breaks in duplex DNA, RNA, or DNA/RNA hybrids, but also join blunt-end and cohesive-end.

Applications

- Blunt or cohesive-end ligation
- Repair of nicks in double-stranded nucleic acids

Components

Components	E-3061	E-3062
T4 DNA Ligase (200 U/µl)	20,000 U (100 µl)	100,000 U (100 µl x 5)
10X Reaction 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 500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 10 mM ATP, and 25 µg/ml BSA, pH 7.8

Storage Buffer

T4 DNA Ligase is supplied in 50% (v/v) glycerol containing 20 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, and 10 mM β-mercaptoethanol, pH 7.5.

Unit Definition

One weiss unit (200 U) is defined as the amount of enzyme required to ligate 90% of *Hind* III fragments of lambda DNA in 30 min at 16°C in total volume of 20 µl.

Quality Control

- Nuclease Contamination Assay: Nuclease activity is not detected after incubation of 1 µg of substrate DNA with 10 weiss units of T4 DNA Ligase in 20 µl reaction volume at 37°C for 18 hrs.

Enzyme Inactivation

T4 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. To minimize the degradation of ATP and DTT, store 10X Reaction buffer in small aliquots.

Online Resources

Korean **English**

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

Ordering Information

Description		Cat. No
T4 DNA Ligase	20,000 U (100 rxn)	E-3061
	100,000 U (500 rxn)	E-3062

Notice

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Explanation of Symbols

Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Thaw reagents</td><td>1. Thaw 10X Reaction buffer and mix thoroughly before use. Then, briefly spin down all components including insert DNA, vector DNA, T4 DNA Ligase, and nuclease-free water.</td></tr>
<tr><td>2</td><td> 
Preparation of reaction mixture</td><td>2. Add insert DNA, vector DNA, 10X Reaction buffer, T4 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
<table><tr><th>Components</th><th>20 µl reaction</th></tr><tr><td>Insert DNA</td><td>Variable</td></tr><tr><td>Vector DNA</td><td>Variable</td></tr><tr><td>10X Reaction buffer</td><td>2 µl</td></tr><tr><td>T4 DNA Ligase (200 U/µl)</td><td>0.1-1 µl</td></tr><tr><td>Nuclease-free water</td><td>Variable</td></tr><tr><td>Total volume</td><td>20 µl</td></tr></table>* **Note**: We recommend the molar ratio of insert DNA : vector DNA = 3 : 1.
3. Mix the reaction mixture by tapping and briefly spin down.</td></tr>
<tr><td>3</td><td> 
Incubate reaction mixture</td><td>4. Incubate the reaction mixture one of the following three conditions.
• Room temperature for 3 hrs
• 4°C for overnights
• 15°C for 4-18 hrs
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.</td></tr>
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