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T4 DNA Ligase

Catalog Number: E101-1, E101-2

Table 1. Kit Components and Storage

Kit Component	E101-1 (250 units)	E101-2 (1250 units)	Storage	Stability
T4 DNA Ligase (5 units/µL)	50 µL	250 µL	-20 °C, avoid repeated free-thaw	The product is stable for at least 6 months when stored as directed.
10× T4 Ligation Buffer	250 µL	1.25 mL		

Product Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. The enzyme repairs single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids, joins DNA fragments with either cohesive or blunt termini. The T4 DNA Ligase requires ATP as a cofactor.

The enzyme is available in 250 and 1,250 unit sizes at a concentration of 5 U/µL. The enzyme is supplied with a 10× T4 Ligation Buffer.

Application

- Routine subcloning.
- Recircularization of linear DNA.
- Library construction.
- Linker ligation.

Product Specifications

- **Storage Buffer:** 10 mM Tris-HCl (pH 7.4 at 25 °C), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, and 50% (v/v) glycerol.
- **10× T4 Ligation Buffer:** 500 mM Tris-HCl (pH 7.4 at 25 °C), 100 mM $MgCl_2$, 10 mM ATP, 100 mM DTT.
- **Unit Definition:** One Weiss unit of the enzyme catalyzes the conversion of 1 nmol of [^{32}PPi] into Norit-adsorbable form in 20 min at 37°C.
- **Enzyme Activity Assay Conditions:** 66 mM Tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 0.066 mM ATP, 10 mM DTT, 3.3 µM [^{32}PPi].
- **Source:** A recombinant E. coli strain carrying the cloned T4 DNA Ligase gene.

General Protocol

1. In a microcentrifuge tube, combine the following reagents for a 20 µL ligation reaction:

10× T4 Ligation Buffer	2 µL
Vector DNA	10-100 ng
Insert DNA	As required*
T4 DNA Ligase	1 U or 5 U**
Nuclease-free water	up to 20 µL

Note: * For cohesive-end ligations, use a 1:1 or a 3:1 molar ratio of insert:vector; for blunt-end ligations, use a 3:1 molar ratio of insert:vector DNA.

** For cohesive-end ligations, use 0.2 µL (1 U) T4 DNA Ligase; for blunt-end ligations, use 1 µL (5 U) T4 DNA Ligase.

2. Mix gently and spin down for a few seconds.
3. Incubate at room temperature for 1 hour.
4. Immediately transform competent cells with 2 µL of the ligation reaction.