T4 DNA Ligase

Cat. No. FL101
Storage: -20℃for one year
Concentration: 200 units/μl

Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA with blunt or cohesive end. The enzyme repairs single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids but has no activity on single-strand nucleic acids. T4 DNA Ligase requires ATP as a cofactor.

Unit Definition

One unit is the amount of enzyme required to give 50% ligation of Hind III fragments of λDNA (5′ DNA termini concentration of 0.12 μM, 200 μg/ml) in a total reaction volume of 20 μl in 30 minutes at 16°C in 1×T4 DNA Ligase Buffer.

Source

E.coli strain carrying T4 DNA ligase gene

Quality Control

Functional absence of endonucleases and exonucleases activities

Applications

• Cloning blunt end or cohesive end fragments.
• Ligation of synthetic linkers or adaptors.

Storage Buffer

10 mM Tris-Cl (pH 7.4), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol

Components

T4 DNA Ligase (200 units/μl), 5×T4 DNA Ligase Buffer [250 mM Tris-Cl (pH 7.5), 50 mM $MgCl_2$, 5 mM DTT, 5 mM ATP, 125 μg/ml BSA, Enhancer]

Reaction Setup

Component	Volume	Final concentration
Vector	Variable	as required
Insert	Variable	as required
5×T4 DNA Ligase Buffer	2 μl	1×
T4 DNA Ligase	0.5-1 μl	100-200 units
ddH_2O	Variable	-
Total volume	10 μl	-

- Cohesive ends ligation: incubate at 25°C for 10 minutes.
- Blunt ends ligation: incubate at 25°C for 2 hours, or overnight at 16°C.
- Cohesive and blunt ends ligation: incubate at 25°C for 2 hours.

Note

It is recommended to use a molar ratio of insert to vector at 3:1-10:1.

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