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T4 DNA Ligase

Cat. Number: E-2010, E-2050

Enzyme Description

This product is a recombinant DNA ligase enzyme from bacteriophage T4. The enzyme has a molecular weight of 55.5 kDa. T4 DNA ligase can join both cohesive and blunt ends by forming a phosphodiester bond between adjacent 5'-phosphate and 3'-hydroxyl ends in double-stranded DNA or RNA fragments. The enzyme can also repair single-strand breaks in duplex DNA. The enzyme requires ATP as a cofactor for activity. Optimal activity is observed at 16°C. The enzyme is inactivated at 65°C for 10 minutes.

Applications

Cloning of restriction fragments.
Joining DNA fragments with blunt ends.

Source

T4 DNA ligase is isolated from *E.coli* strain containing a plasmid with the cloned gene of T4 bacteriophage enzyme.

Unit Definition

One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (300 ng/μl) in a total reaction volume of 20 μl in 30 minutes at 16°C in Standard Reaction Buffer.

Enzyme concentration and packaging: 200 U/μl.

Cat. No.	Product Name	Quantity	Volume
E-2010	T4 DNA Ligase	10 000 U	50 μl
E-2050		50 000 U	250 μl

Storage Buffer: 10 mM Tris-HCl (pH 7.5 at 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, and 50% glycerol.

Quality Control: Each batch of the enzyme is tested for specific activity, electrophoretic purity in SDS-PAGE, and DNase activity.

Standard 1x Reaction Buffer: 50 mM Tris-HCl (pH 7.5 at 25°C), 10 mM $MgCl_2$, 1 mM ATP, 10 mM DTT (500 μL of 10x buffer is supplied with the enzyme).

Typical reaction conditions

For cloning DNA fragments, mix the following components in a tube:

- 2 μl of 10x Standard Reaction Buffer;
- 40-50 ng vector DNA (4-5 kb);
- 30-40 ng insert DNA (800-1000 bp);
- up to 18 μl of nuclease-free water;
- 2 μl (400 units) T4 DNA ligase.

Incubate the reaction mixture at 16°C overnight.

Storage and transportation conditions: Store at -20°C. Transportation at temperatures not exceeding +8°C is allowed for up to one day.