Tth DNA Ligase

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Tth DNA Ligase

Tth DNA Ligase catalyzes the NAD-dependent formation of phosphodiester bonds between adjacent 3' hydroxyl and 5'-phosphate termini in double stranded DNA. It is not active against single stranded DNA or RNA and blunt ended DNA. Enzyme is isolated from *Escherichia coli* strain containing plasmid carrying the *Thermus thermophilus* DNA ligase gene. **Tth DNA Ligase** is stable and active in optimum ligation temperature range of 45–65°C, which is 7–10°C higher than that of T4 DNA ligase. The final reaction ligation temperature is determined by the Tm of the substrates. High ligation temperature eliminates the nonspecific ligation.

Features and advantages

- → Stable and active in high temperatures
- → Highly specific ligation
- → LCR (Ligase Chain Reaction)
- → LDR (Ligase Detection Reaction)
- → NGS (Next-Generation DNA Sequencing)
- → RED (Repeat Expansion Detection)
- → RCA (Rolling Circle Amplification)
- → PLA (Proximity Ligation Assay

Note: Some applications in which this product may be used may be covered by patents or patent applications applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a license depending upon the particular application and country in which the product is used.

Stability

Enzyme retains full activity after incubation for 1 week at 37°C. The half-life of enzyme is about 48 hours at 65°C. 10x Tth Ligation Buffer is stable for 1 week at 37°C. Up to tweenty freeze/thaw cycles will not compromise 10x Tth Ligation Buffer performance.

Protocol

1. Add the reaction reagents listed below to a sterile nuclease-free tube. The reaction agents should be added in the following order:

Component	Volume
Nuclease-free water	up to 25 µl
10x Tth Ligation Buffer	2.5 µl
Tth DNA Ligase 5 U*/µl (75 CEU/µl)	0.5–1 µl
DNA	0.5–1 µg

2. Mix gently and spin briefly.
3. Incubate for 10 min at 45–65°C. Optimum ligation temperature range is determined by the Tm of the substrates.

Quality control

Tth DNA Ligase activity is tested in reaction with bacteriophage lambda DNA digested with SalI and SmaI, with a dilution series of ligase. Results are assayed by agarose gel electrophoresis. Free of unspecific DNA and RNA nucleases contamination.

Storage Buffer

50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100, 1 mM DTT, 50% glycerol

10x Tth Ligation Buffer

200 mM Tris-HCl (pH 8.3), 250 mM KCl, 100 mM $MgCl_2$, 5 mM NAD, 0.1% Triton X-100

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Components	EN13-025 250 U (3750 CEU)	EN13-250 2500 U (37 500 CEU)	EN13-S 25 U (375 CEU)
Tth DNA Ligase 5 U*/µl (75 CEU/µl)	50 µl	500 µl	5 µl
10x Tth Ligation Buffer	125 µl	1250 µl	12.5 µl

* Unit definition may vary between manufacturers

Additional information

Unit definition

One unit of Tth DNA Ligase catalyzes the ligation of 50% of the cos sites present in 1 µg of bacteriophage lambda DNA in 1 minute at 45°C.

1 U (Unit) of Tth DNA Ligase = **1 Ampligase® Unit** = **15** cohesive end units (**CEU**).

Storage conditions

All components should be stored at -20°C.
Up to twenty freeze/thaw cycles will not compromise 10x Tth Ligation Buffer performance.

Shipping conditions

Shipping on dry or blue ice.

For research use only

Expiry

Information on the label.

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