

July 2024

Tth DNA Ligase

Tth DNA Ligase (cat. nos. EN13-025, EN13-250, and EN13-B) 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 *E. 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 T_m (melting temperature) of the substrates. High ligation temperature eliminates nonspecific ligation.

The Tth DNA Ligase and its components should be shipped on dry ice and stored at –20°C.

Further information

- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: [support.qiagen.com](mailto:support@qiagen.com)

Notes before starting

- Enzyme retains full activity after incubation for 1 week at 37°C.
- The half-life of the enzyme is about 48 hours at 65°C.
- 10x Tth Ligation Buffer is stable for 1 week at 37°C.

- Up to 20 freeze/thaw cycles will not compromise 10x Tth Ligation Buffer performance.
 - The concentration of the enzyme is 5 U/μL.
 - 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 min at 45°C.
- Note:** 1 U (Unit) of Tth DNA Ligase is equivalent to 15 cohesive end units (CEU)

Protocol

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

Table 1. Reagents to be added and corresponding quantities

Component	Quantity
Nuclease-free water	Up to 25 μL
10x Tth Ligation Buffer	2.5 μL
Tth DNA Ligase	0.5–1 μL
DNA	0.5–1 μg

2. Mix gently and spin briefly.
3. Incubate for 10–30 min at 45–65°C. Optimum ligation temperature range is determined by the *T*_m of the substrates.

Document Revision History

Date	Changes
08/2023	Initial release
07/2024	Added the mention of catalog number EN13-B.

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