August 2023

Quick-Start Protocol

# Tth DNA Ligase

Tth DNA Ligase (cat. nos. EN13-025, EN13-250) 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**

## 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 minute 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 min at 45–65°C for 30 min. Optimum ligation temperature range is determined by the *T*m of the substrates.

# Document Revision History

| Date | Changes |
|---|---|
| August 2023 | Initial release |

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