T4 DNA Ligase

Cat. No. G467

Store at -20°C.

Product Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the proximal 3'-hydroxyl and 5'- phosphate ends of adjacent, nicked nucleotides of dsDNA, dsRNA or DNA-RNA hybrids. Known to proceed via an adenylated intermediate, this endergonic reaction can be utilized to join both cohesiveended and blunt-ended termini.

Product Component	Quantity	Part No.
T4 DNA Ligase (400 U/µl)	200 µl	G467-1
5X T4 DNA Ligase Reaction Buffer	1 ml	G467-2

Product Applications

- Cloning of restriction fragments
- Attaching double-stranded oligonucleotide linkers or adaptors to DNA
- Site-directed mutagenesis
- Nick repair of duplex DNA, RNA or DNA-RNA hybrids
- Self-circularization of linear DNA

Protocol

1. Add the following components to a sterile tube, add the T4 DNA Ligase last:

Product Component	Volume
5X T4 DNA Ligase Buffer	4 µl
Vector DNA*	variable
Insert DNA*	variable
T4 DNA Ligase (400 U/µl)	1 µl
Nuclease-Free H_2O	up to 20 µl

*Note: A molar ratio of 1:3 vector to insert is recommended. To calculate the amount of insert and vector needed, use the following formula:

$$Mass\ of\ Insert\ (ng) = Desired\ Molar\ Ratio\ \frac{Insert}{Vector} \times Mass\ of\ Vector\ (ng) \times \frac{kb\ size\ of\ insert}{kb\ size\ of\ vector}$$

2. Pipette up and down to gently mix the components and then briefly centrifuge.
3. **For sticky ends:** Incubate at 16°C overnight or at room temperature for 10 minutes.
   For blunt ends or single base overhangs: Incubate at 16°C overnight or at room temperature for 2 hours.
4. Once the reaction is completed, chill on ice and then transform 1-5 µl into 30 -50 µl of competent cells or store at -20°C for long term storage.

General Notes

- For heat inactivation, 65°C for 10 minutes or 70°C for 5 minutes
- Store all components at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for one year from the date of shipping when stored and handled properly.
- One unit is the enzyme quantity needed to achieve 50% ligation of HindIII fragments of λ DNA (with 5´ DNA termini concentration of 0.12 µM, and 300 µg/ml) in a 20 µl total reaction volume, within 30 minutes at 16°C in 1X T4 DNA Ligase Reaction Buffer.

Applied Biological Materials Inc.● 1-866-757-2414 ● info@abmGood.com ● www.abmGood.com