T4 DNA Ligase

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T4 DNA Ligase is an ATP-dependent recombinant enzyme isolated from *Escherichia coli* strain used to clone DNA. **T4 DNA Ligase** catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. It will join both blunt-ended and cohesive-ended restriction fragments of DNA, as well as repair single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

Applications

- → Cloning PCR products
- → Cloning restriction fragments
- → Joining double-stranded oligonucleotide linkers or adaptors to DNA
- → Site-directed mutagenesis
- → Nick repair in duplex DNA, RNA or DNA/RNA hybrids
- → Self-circularization of linear DNA
- → LM PCR methods (Ligation Mediated PCR), e.g. amplified fragment length polymorphism (AFLP)

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Protocol

1. Add the reaction reagents listed below to a sterile nuclease-free Eppendorf tube placed **on ice in a freezing rack**. The reaction agents should be added in the following order:

Component	Volume
Vector DNA	x µl (20 – 50 ng)
Insert DNA	y µl (3 – 10 molar excess over vector)*
10x T4 Ligation Buffer	2 µl
ATP Solution (25 mM)	0.4 – 0.8 µl
T4 DNA Ligase (5 U/µl)	1 µl
Nuclease-free water	up to 20 µl

* A lower ratio will result in a less efficient ligation; a higher ratio will incite multiple insertions. This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

2. Mix gently and spin briefly.
3. For cohesive (sticky) ends, incubate at 20-25°C for 0.5-1 hour. For blunt ends incubate at 20-25°C for 1-2 hours.
4. Cool the samples on ice and transform 1-5 µl of the reaction mixture into 50 µl *E. coli* competent cells.

Additional information

- The 10x T4 Ligation Buffer and ATP Solution should be thawed and resuspended at room temperature.
- For blunt-end ligations, use higher quantities of both the vector and the insert DNA.
- For sticky (cohesive)-end ligations, we recommend heating both the vector and the insert DNA prior to ligation.
- The electrotransformation efficiency may be improved by heat inactivation of the **T4 DNA Ligase** and purification of the DNA by means of a spin column purification method (***EXTRACTME* DNA CLEAN-UP** kit or ***EXTRACTME* DNA GEL-OUT** kit).
- We recommend using a 3–10 molar excess of insert DNA over vector DNA. To calculate the optimal amounts of insert DNA in a ligation reaction, use the following equation:

$$\text{ng of insert} = \frac{\text{ng of vector} \times \text{kb size of insert} \times \text{molar ratio insert:vector}}{\text{kb size of vector}}$$

Example: If using 20 ng of a vector plasmid (4 kb), for a 5:1 molar ratio of insert:vector, you will require the following quantity of 1 kb insert:

$$\frac{20 \times 1 \times 5}{4} = 25 \text{ ng}$$

- The enzyme is inhibited by >200 mM NaCl or KCl concentrations.
- Inactivate enzyme at 65°C for 10 minutes or at 70°C for 5 minutes.

Storage buffer

10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 50 mM KCl, 50% (v/v) glycerol

10x T4 Ligation Buffer

660 mM Tris-HCl (pH 7.5), 100 mM DTT, 100 mM $MgCl_2$

The 10x T4 Ligation Buffer does not contain ATP, which must be added separately. For most applications, ATP should be added to the reaction to a final concentration of 0.5–1.0 mM. 25 mM ATP Solution is included.

Quality control

The product was tested in ligation of *Hind*III-cut lambda DNA with a different amounts of enzyme. The product is free of unspecific DNA nucleases.

Unit definition

One (Weiss) unit of **T4 DNA Ligase** catalyzes the conversion of 1 nmol of ^{32}P from pyrophosphate into Norit-adsorbable material in 20 minutes at 37°C.

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Components	EN11-050 500 U	EN11-250 2500 U
T4 DNA Ligase 5 U/µl	100 µl	5x 100 µl
10x T4 Ligation Buffer	200 µl	5x 200 µl
ATP Solution (25 mM)	80 µl	5x 80 µl

Storage & shipping

Storage conditions
All components should be stored at -20°C.

Shipping conditions
Shipped on dry or blue ice.

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