T4 DNA Ligase

User Manual | Recombinant, Research Grade

Cat. No. EV-MOL-010 | Version 1.0 | April 2026

Cat. No.	EV-MOL-010	**Size**	10,000 units | 100,000 units	**Storage**	–20°C

1. Overview

T4 DNA Ligase (EV-MOL-010) is a recombinant, research-grade enzyme encoded by gene 30 of the T4 bacteriophage. It is a monomeric polypeptide of 487 amino acids with a molecular weight of approximately 55.3 kDa. The enzyme catalyzes the formation of phosphodiester bonds between juxtaposed 5′-phosphate and 3′-hydroxyl termini in duplex DNA in an ATP-dependent manner, making it the most widely employed ligase in molecular biology.

The Enzovera EV-MOL-010 formulation is produced in *E. coli* expression systems and purified to >95% homogeneity by multi-step chromatography. Each lot is activity-tested and quality-controlled for nuclease contamination prior to release.

2. Catalytic Mechanism

The ligation reaction proceeds through three sequential steps, all strictly requiring ATP and Mg^{2+}:

Step 1 — Enzyme Adenylation: T4 DNA Ligase reacts with ATP to form a covalent enzyme-AMP intermediate via attachment to Lysine-159.

Step 2 — Adenylate Transfer: The AMP moiety is transferred to the 5′-phosphate of the donor DNA strand, activating it for nucleophilic attack.

Step 3 — Nick Sealing: The 3′-OH of the recipient strand attacks the activated 5′-phosphate, displacing AMP and forming the phosphodiester bond.

3. Substrate Specificity

Substrate Type	Description	Notes
Cohesive (Sticky) Ends	Compatible 4-base 5′ or 3′ overhangs	Standard conditions; highly efficient
Blunt Ends	Flush-ended duplex DNA	Requires higher enzyme concentration; 16°C recommended
Nicked dsDNA	Single-strand break in dsDNA	Effective for nick repair in plasmid substrates
RNA/DNA Hybrids	Nicked RNA strand in DNA:RNA duplex	Lower efficiency; requires optimization

4. Reaction Conditions & Protocol

4.1 Recommended Reaction Buffer

Component	Final Concentration
Tris-HCl (pH 7.5)	50 mM
MgCl■	10 mM
DTT	10 mM
ATP	1 mM
BSA (optional)	25 µg/mL

4.2 Standard Ligation Protocol (20 µL)

Component	Volume
Insert DNA (3:1 molar ratio)	x µL
Vector DNA (linearized)	y µL
10× T4 DNA Ligase Buffer	2 µL
T4 DNA Ligase (EV-MOL-010)	1 µL (400 units)
Nuclease-free water	to 20 µL

 Incubation Conditions:
• Cohesive ends: 16°C for 16 hours OR 25°C for 5 minutes (Quick Ligation mode)
• Blunt ends: 16°C for 16 hours (400 units/reaction)
• Inactivation: 65°C for 10 min or 70°C for 5 min

5. Unit Definition

One unit (U) is defined as the amount of enzyme required to give >95% ligation of 1 µg of HindIII-digested lambda DNA (cohesive ends) in 30 minutes at 16°C in a total reaction volume of 20 µL.

6. Quality Control

Test	Specification
Purity (SDS-PAGE)	>95% (single band at ~55.3 kDa)
Ligation Activity	>95% of HindIII-cut λDNA ligated (16°C, 30 min)
Exonuclease Activity	No detectable activity (200 U, 4 h, 37°C)
Endonuclease Activity	No nicking of supercoiled pUC19 (200 U, 4 h, 37°C)
RNase Activity	No detectable degradation (200 U, 2 h, 37°C)

7. Storage & Stability

- **Storage temperature:** −20°C (avoid repeated freeze-thaw; aliquot upon receipt)
- **Supplied in:** 10 mM Tris-HCl (pH 7.4), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/mL BSA, 50% glycerol
- **Stability:** 24 months from date of manufacture when stored correctly
- **Shipping:** On dry ice

8. Applications

- Routine cloning: ligation of PCR products or restriction fragments into vectors
- Library construction: adapter ligation for Next-Generation Sequencing (NGS)
- Linker/adapter ligation: adding defined sequences to blunt-ended DNA
- Site-directed mutagenesis: sealing nicks in circular plasmids after primer extension
- Gibson Assembly supplemental step (nick sealing)

9. Troubleshooting

Problem	Possible Cause	Suggested Action
No ligation	ATP depleted or absent	Ensure 1 mM ATP in buffer; avoid repeated freeze-thaw of buffer
No ligation	Incompatible ends	Verify end types are compatible; use blunt-end protocol if needed
Low efficiency	Suboptimal insert:vector ratio	Optimize molar ratio (typically 3:1 to 5:1 insert:vector)
High background colonies	Incomplete vector dephosphorylation	Treat vector with CIP/SAP before ligation
Concatemers	Excess insert DNA	Reduce insert concentration or use diluted ligation for transformation

10. Safety Information

This product is intended for research use only. Handle in accordance with standard laboratory safety guidelines. Refer to the accompanying Safety Data Sheet (SDS) for full hazard information. Avoid ingestion, inhalation, or contact with eyes and skin. Dispose of in accordance with local, state, and federal regulations.