T4 DNA Ligase

Product Information

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Kit contents

Component	ATR-C801 (20,000 Units)
T4 DNA Ligase (400 U/µL)	50 µL (20,000 U)
10X T4 DNA Ligase Buffer	500 µL
50% PEG 6000 Solution	300 µL

Product Description

ATR-MED® T4 DNA Ligase is a highly purified, recombinant enzyme derived from the bacteriophage T4 gene, engineered for ATP-dependent catalysis of phosphodiester bond formation between adjacent 5'-phosphate and 3'-hydroxyl termini in double-stranded DNA or RNA. This enzymatic activity is essential for covalent joining of nucleic acid ends, making it a cornerstone for molecular biology techniques such as restriction fragment cloning, cohesive-end and blunt-end ligation, PCR product insertion, TA cloning, site-directed mutagenesis, adapter or linker ligation, and repair of single-strand nicks in DNA, RNA, or DNA/RNA duplexes. Benchmarked against leading commercial ligases (e.g., New England Biolabs® and Thermo Fisher Scientific®), ATR-MED® T4 DNA Ligase demonstrates comparable or superior performance across diverse ligation protocols. In blunt-end ligation assays, which are inherently challenging, the enzyme achieves up to 7-fold higher ligation efficiency. For cohesive-end ligation and TA cloning, it significantly enhances transformation efficiency, establishing it as a high-performance solution for both routine and high-throughput applications. The inclusion of a 50% PEG 6000 solution enhances blunt-end ligation efficiency, broadening its utility. Manufactured under a tightly controlled recombinant expression system in *Escherichia coli*, the enzyme ensures ≥95% purity (verified by SDS-PAGE) and exceptional lot-to-lot consistency. It supports flexible protocols, including rapid ligation (10–30 minutes at 22–25°C) and standard overnight incubations at 16°C, with broad compatibility across various PCR and restriction enzyme buffers. ATR-MED® T4 DNA Ligase is optimized for academic research, synthetic biology, and next-generation sequencing (NGS) library preparation.

Applications

- Cloning of restriction enzyme-digested DNA fragments (cohesive-end ligation)
- Blunt-end ligation of PCR or restriction products
- TA cloning of PCR-amplified DNA fragments
- Ligation of double-stranded adapters and linkers
- Circularization of linear DNA molecules
- Site-directed mutagenesis workflows
- Repair of nicks in double-stranded DNA, RNA, or DNA/RNA hybrids

Highlights

- **High Efficiency**: Up to 7-fold improvement in blunt-end ligation and enhanced efficiency in TA and cohesive-end cloning
- **Flexible Protocols**: Supports rapid (10–30 min at 22–25°C) or standard (16°C, 2 h–overnight) incubations
- **Broad Buffer Compatibility**: Retains 75–100% activity in various commercial PCR and restriction enzyme buffers supplemented with 0.5 mM ATP
- **Versatility**: Suitable for sticky-end, blunt-end, TA, and adapter/linker ligations
- **Reproducibility**: Manufactured under stringent conditions for consistent activity and lot-to-lot reliability

Source

Recombinantly expressed and purified from an *Escherichia coli* strain harboring the cloned T4 DNA ligase gene.

Unit Definition

One Cohesive-End Unit (CEU) is defined as the amount of enzyme required to ligate 50% of HindIII-digested λ DNA termini (0.12 µM ends; 300 µg/mL DNA) in a 20 µL reaction at 16°C for 30 minutes in 1X T4 DNA Ligase Buffer. Conversion: 1 Weiss Unit ≈ 200 CEU.

Buffer Composition

- **Storage Buffer**: 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% (v/v) glycerol.
- **10X T4 DNA Ligase Buffer**: 400 mM Tris-HCl (pH 7.8 at 25°C), 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP.
- **50% PEG 6000 Solution**: Used at 5% (v/v) final concentration to enhance blunt-end ligation efficiency.

Storage

Store all components at -20°C to maintain stability. Avoid repeated freeze-thaw cycles. Aliquot the 10X T4 DNA Ligase Buffer to prevent ATP degradation, which may reduce ligation efficiency.

Shipping

Shipped on gel ice packs to ensure stability. Upon receipt, transfer all components promptly to a -20°C freezer.

1. Protocol

1.1. Sticky-end ligation

1. Prepare the following 20 µL reaction mixture on ice:

Component	Amount
Vector DNA	50–200 ng
Insert DNA*	Adjust for molar ratio
10X Ligation Buffer#	2 µL
T4 DNA Ligase (400 U/µL)	1 µL (400 U)
Nuclease-Free Water	To 20 µL

* Total DNA concentration (vector + insert) should be 1–10 µg/mL. Recommended vector:insert molar ratios are 1:1 to 1:10 (1:3 typical). Perform multiple ligations with varying ratios if DNA concentrations are uncertain.
# Invert and mix 10X T4 DNA Ligase Buffer well before use, as slight precipitation may occur upon thawing.

2. Gently mix, spin down, and incubate:
   - **Standard**: 16°C overnight for maximum yield.
   - **Rapid**: 22–25°C for 10–30 minutes.
3. For transformation, use 1–5 µL in 50 µL chemically competent cells or 1–2 µL (post-purification) in electrocompetent cells.

1.2. Blunt-end ligation

1. Prepare the following 20 µL reaction mixture on ice:

Component	Amount
Vector DNA	50–100 ng
Insert DNA*	Adjust for molar ratio
10X Ligation Buffer	2 µL
50% PEG 6000 Solution **(Optional)**	2 µL (5% final)
T4 DNA Ligase (400 U/µL)	1 µL (400 U)
Nuclease-Free Water	To 20 µL

*Recommended vector:insert molar ratio is 1:5 to 1:10.

2. Gently mix, spin down, and incubate:
   - **Standard**: 16°C overnight for maximum yield.
   - **Rapid**: 22–25°C for 2 hours.

3. For transformation, use 1–5 µL in 50 µL chemically competent cells or 1–2 µL (post-purification) in electrocompetent cells.

1.3. Self-Circularization of Linear DNA

1. Prepare the following 50 µL reaction mixture on ice:

Component	Amount
Linear DNA	10–50 ng
10X Ligation Buffer	5 µL
T4 DNA Ligase (400 U/µL)	1 µL (400 U)
Nuclease-Free Water	to 50 µL

2. Gently mix, spin down, and incubate:

   - **Sticky-end ligation**: 16°C for 2 hours to overnight (standard) or 22–25°C for 10–30 minutes (rapid).
   - **Blunt-end ligation**: 16°C overnight (standard) or 22–25°C for 2 hours (rapid).

3. For transformation, use 1–5 µL in 50 µL chemically competent cells or 1–2 µL (post-purification) in electrocompetent cells.

1.4. Linker Ligation

1. **Prepare Linker**: Dissolve linker in 100 µL nuclease-free water to achieve 60 pmol/µL. Store at -20°C or keep on ice.

2. **Ligation Reaction Setup**:
   - Precipitate DNA fragment(s) using ethanol precipitation.
   - Resuspend DNA in 10 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
   - Add phosphorylated linker (50–100× molar excess relative to DNA ends; ~1–2 µg linker per 100 ng DNA).
   - Add 2.5 µL 10X T4 DNA Ligase Buffer.
   - Adjust to 24 µL with nuclease-free water.
   - Add 1 µL T4 DNA Ligase (400 U/µL).
   - Mix gently, spin down, and incubate at 16°C overnight.

3. **Post-Ligation Processing**:
   - Heat-inactivate ligase at 75°C for 5 minutes, then cool on ice.
4. **Restriction Digestion of Linkers**:
   - Adjust salt concentration if required (e.g., add 5 mM NaCl for high-salt enzymes).
   - Add 1–2 units of restriction enzyme per pmol of linker.
   - Adjust to 30 µL with nuclease-free water and incubate at the enzyme's optimal temperature for 4 hours.

5. **Removal of Excess Linker**:

- **Option 1: Spin Column** – Use a G-25 spin column for size exclusion, following manufacturer instructions.
- **Option 2: Ultrafiltration** – Dilute ligation mixture to 2 mL with deionized water in a Centricon 30/50 unit, centrifuge per specifications, repeat wash, and recover concentrate by inverting and spinning at 300–1,000 × g for 2 minutes.
- **Option 3: Low-Melt Agarose Gel** – Run ligation products on low-melt agarose gel, excise the correct band, and purify using a gel extraction kit.
- **Option 4: PEG Precipitation** – Use polyethylene glycol to selectively precipitate larger DNA fragments.
- **Notes**: Use fresh ligase and buffer, work on ice, verify ligation via gel shift if needed, and supplement restriction enzyme buffers with 0.5 mM ATP for linkers with restriction sites.

2. Vector:Insert Molar Ratio Formula

To calculate the required insert DNA mass:

Insert mass (ng)=Vector mass (ng) × (Insert size (bp)/Vector size (bp)) × Desired molar ratio

Example:

- Vector: 5,000 bp, 50 ng
- Insert: 1,500 bp
- Ratio: 3:1
- Insert = 50 × (1500/5000) × 3 = **45 ng**

3. Recommended Molar Ratios:

Ligation Type	Recommended Molar Ratio (Insert:Vector)	Notes
Sticky-End Ligation	3:1	Standard and effective
Blunt-End Ligation	5:1 to 10:1	Higher ratio enhances efficiency
TA Cloning	3:1	Ensure proper A-tailing
Adapter/Linker	10:1 or greater	Use excess linker for efficiency

4. Transformation

1. Thaw 50 µL chemically competent *E. coli* (e.g., DH5α) on ice.
2. Add 1–2 µL ligation mixture (~5 ng DNA, ≤10% cell volume). Mix gently by pipetting or flicking; do not vortex.
3. Incubate on ice for 30 minutes without mixing.
4. Heat shock at 42°C for 45–90 seconds, then return to ice for 2 minutes.
5. Add 950 µL SOC or LB medium (without antibiotics) and incubate at 37°C for 1 hour with shaking (200–250 rpm).
6. Preheat selective LB agar plate to 37°C.
7. (Optional) Pellet cells at 2,400 × g for 5 minutes and resuspend in a smaller volume before plating.
8. Plate 50–100 µL on selective agar and incubate overnight at 37°C.
9. Follow competent cell manufacturer's recommendations for heat shock duration and recovery medium.

5. Electrotransformation and Special Notes

- **Improving Electrotransformation**:
  - Heat-inactivate T4 DNA Ligase at 65°C for 10 minutes (or 70°C for 5 minutes) before electroporation.
  - Purify ligation mixtures using spin columns (e.g., PCR Purification Kit) or chloroform extraction to prevent arcing.
- **Enhancing Yield**: Extend post-ligation incubation to 1 hour at 22–25°C to increase transformants.
- **High Enzyme Caution**: If using >400 U in a 20 µL reaction, purify DNA before electroporation to avoid arcing.

6. Compatibility with Common Buffers

ATR-MED® T4 DNA Ligase retains substantial activity in various commercial buffers when supplemented with 0.5 mM ATP, enabling integration into multi-step workflows without buffer exchange.

Buffer Type	Representative Buffers	Relative Activity (%)*
PCR Buffers	*Taq* (KCl), *Taq* $(NH_4)_2SO_4$, *Pfu*, Reverse Transcriptase	~75%
Restriction Enzyme Buffers	FastDigest™, Tango™, and NEBuffer™	75–100%
Low-Compatibility Buffers	*Ecl136II*, *PacI*, *SacI*	~50%

* Activity measured with 0.5 mM ATP supplementation. Optimal performance is achieved with the supplied 10X T4 DNA Ligase Buffer.

7. Troubleshooting

- **Low Colony Yield**: Verify DNA integrity (A260/280 ~1.8–2.0) and concentration. Extend ligation to 24 hours at 16°C and use a 3:1 insert-to-vector molar ratio.
- **High Background (Empty Vectors)**: Dephosphorylate vector ends with alkaline phosphatase and gel-purify vector/insert to remove uncut or circularized plasmids.
- **Smearing/Ghost Bands on Gels**: Use freshly purified DNA (A260/230 >1.8), minimize UV exposure during gel extraction, and include a cleanup step (e.g., spin-column or ethanol precipitation).
- **Failed PCR from Transformants**: Purify plasmid DNA via miniprep or spin-column, design junction-specific primers, and limit template to <10 ng per reaction to reduce inhibitors.
- **Weak Blunt-End Ligation**: Optimize PEG 6000 to 5–10% (v/v) and increase ligase to 800 U (2 µL) per 20 µL reaction. Extend incubation to 24 hours at 16°C.
- **Poor Transformation Efficiency**: Heat-inactivate ligase (65°C, 10 min or 70°C, 5 min), purify DNA, use ≤10% ligation volume in competent cells, and confirm cell competency (>1×10^8 CFU/µg).

Precautions and Disclaimer

This product is designated for research and development purposes only and is not intended for therapeutic, diagnostic, household, or other non-research applications. Handle using standard laboratory protective equipment, including lab coats, disposable gloves, and safety goggles. When using radioactive nucleotides, adhere to institutional radiation safety protocols. Comprehensive safety data are available in the Material Safety Data Sheets (MSDSs) at www.atrmed.com or via email request to info@atrmed.com. To the maximum extent permitted by applicable law, ATR-MED Inc. disclaims liability for special, incidental, indirect, punitive, or consequential damages arising from the use of this product or associated documentation. Product use constitutes acceptance of ATR-MED's terms and conditions. All trademarks are owned by ATR-MED unless otherwise specified.

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