T4 DNA Ligase

Cat. No.: T665705 | Pack size: 40 KU / 5 x 40 KU | Storage: -20 °C; avoid repeated freeze-thaw

Overview

T4 DNA Ligase catalyzes phosphodiester bond formation between 5-phosphoryl and 3-hydroxyl ends of double-stranded DNA or RNA with cohesive or blunt ends. ATP is required as a cofactor.

The enzyme is commonly used for ligation of DNA fragments to vectors, linkers, and adaptors, for nick repair, and for ligase-mediated RNA detection workflows.

Key Features

- Recombinant T4 DNA Ligase supplied at 1000 U/µL
- Ligation of cohesive and blunt-ended DNA/RNA duplex substrates
- Repairs single-strand nicks in double-stranded DNA, RNA, and DNA/RNA hybrids
- Supplied with 10x Ligation Buffer
- Free of detectable DNA endonucleases, exonucleases, phosphatases, and RNases

Product Specifications

Parameter	Specification	Notes
Product name	T4 DNA Ligase	CAS No. 9015-85-4; accession P00970
Cat. No.	T665705	Available pack sizes: 40 KU and 5 x 40 KU
Source	Recombinant expressed in Escherichia coli	Sterile liquid
Enzyme concentration	1000 U/µL	Bioactivity: 1000 U/µL
Storage buffer	20 mM Tris pH 7.5, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol	Keep on ice during setup
Unit equivalence	200 U is equivalent to 1 Weiss unit	Per activity definition

Product Contents & Storage

Cat. No.	Component	40 KU	5 x 40 KU	Storage
T665705A	T4 DNA Ligase (1000 U/μL)	40 μL	5 x 40 μL	-20 ℃; avoid freeze-thaw
T665705B	10x Ligation Buffer	300 μL	5 x 300 μL	-20 ℃; avoid freeze-thaw

Shelf life: Stable for 24 months at -20 ℃.

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Vector DNA and insert DNA	Purified, compatible ends	Ligation substrate
Nuclease-free water	DNase/RNase-free	Reaction volume adjustment
Restriction enzymes and purification kit	User-supplied	Vector/insert preparation
Competent cells	Application-specific	Transformation after ligation
Thermal incubator	16 ℃ and 20-25 ℃ capability	Ligation incubation

Preparation Before Use

1. Thaw 10x Ligation Buffer on ice and mix thoroughly until homogeneous.
2. Use purified vector and insert DNA. For conventional cloning, a 3:1 insert:vector molar ratio is recommended as a starting point.
3. Keep T4 DNA Ligase on ice during setup and add enzyme last.

Protocol

Recommended Reaction Setup

Reagent	Typical Volume / Amount
Vector	approximately 50-100 ng
Insert fragment	approximately 3x molar amount relative to vector

Reagent	Typical Volume / Amount
10x Ligation Buffer	2 µL
Double-distilled water or Milli-Q water	add to 20 µL
T4 DNA Ligase	0.2-0.5 µL
Total volume	20 µL

1. Digest and purify vector DNA. Purify PCR products or insert fragments after gel extraction or appropriate cleanup.
2. Set up the 20 µL ligation reaction using the table below. For blunt-end ligation, longer incubation is required.
3. Mix gently by pipetting or brief vortexing, then briefly centrifuge to collect liquid at the bottom of the tube.
4. Incubate at 20-25 °C for 1-2 h for routine ligation or at 16 °C overnight. For blunt-blunt ligation, overnight incubation is recommended.
5. Use ligation product directly for chemical transformation, or purify before electroporation if required.
6. If gel electrophoresis analysis is required, incubate at 65 °C for 10 min first to reduce band shift caused by ligase binding.

Expected Results

Expected Outcome	Description
Vector-insert ligation	Formation of covalently joined DNA molecules suitable for transformation or downstream analysis

Safety & Precautions

- For routine E. coli transformation, purification of the ligation product is usually unnecessary; purification is recommended before electroporation.
- For blunt-end ligation or rapid ligation, a rapid DNA ligation kit may provide higher efficiency.
- T4 DNA Ligase can be inactivated at 65 °C if analysis requires removal of ligase-DNA binding effects.
- Use a plasmid transformation control and unligated vector control when troubleshooting cloning.
- Wear a lab coat and disposable gloves during operation.

Read the current SDS before use. Wear lab coat, gloves, and eye protection. Handle reagents according to local laboratory safety and EHS procedures.

Quality Control

QC Item	Method	Acceptable Range
Component completeness	Visual inspection on receipt	All enzyme, buffer, and accessory components match the Product Contents & Storage table.
Functional activity	Application-specific ligation, digestion, or amplification assay	Product performs at the expected unit concentration and produces the expected reaction product.
Nuclease contamination	Control incubation followed by gel or fluorometric analysis	No detectable contaminating nuclease activity for the intended application.

Troubleshooting

Issue	Possible Causes	Corrective Action
Low or no product formation	• Enzyme inactive due to improper storage • Missing required cofactor or buffer component • Reaction temperature/time not optimized	• Keep enzyme on ice during setup and store at -20 °C • Use the supplied buffer and verify ATP/NAD/Mg2+ requirements • Optimize enzyme amount, incubation time, and temperature
Poor downstream transformation or assay signal	• Excess salt, EDTA, PEG, or enzyme carried into downstream assay • Substrate quality or molar ratio not optimized	• Purify products when required, especially before electroporation • Use clean substrate and optimize vector:insert or donor:acceptor ratios
Non-specific or unexpected products	• Substrate impurities or incomplete digestion • Reaction over-incubation	• Purify substrates and confirm digestion/annealing quality • Shorten incubation or reduce enzyme amount

Recommended Applications

DNA fragment-to-vector ligation, linker/adaptor ligation, T-vector cloning, self-circularization, nick repair, and ligase-mediated RNA detection research workflows.

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, use the North American sales and customer service contacts below.

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Limitations & Disclaimer

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Where any reagent or component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS), product label, and approved specifications take precedence over this document for handling, storage, transport, and disposal.

Performance depends on sample type, sample condition, handling, storage, and operator technique. Users are responsible for validating this product for their specific application.