T3 DNA Ligase

Cat. No.: T750867 | Pack size: 150 KU / 600 KU / 5 x 600 KU | Storage: -20 °C; avoid repeated freeze-thaw

Overview

T3 DNA Ligase is an ATP-dependent double-stranded DNA ligase derived from T3 bacteriophage and produced recombinantly. It catalyzes cohesive-end and blunt-end DNA ligation and nick repair in DNA or DNA-RNA hybrid duplexes.

T3 DNA Ligase exhibits higher salt tolerance than T4 DNA Ligase and retains high activity in the presence of elevated NaCl or KCl, making it useful for ligation under high ionic strength conditions.

Key Features

- Recombinant T3 DNA Ligase supplied at 3 KU/µL
- Efficient ligation of cohesive-ended and blunt-ended double-stranded DNA
- Supports nick repair in DNA or DNA-RNA hybrid duplexes
- High NaCl/KCl tolerance relative to T4 DNA Ligase
- Supplied with 2x Reaction Buffer containing ATP and PEG 6000

Product Specifications

Parameter	Specification	Notes
Product name	T3 DNA Ligase	CAS No. 9015-85-4; accession P07717
Cat. No.	T750867	Available pack sizes: 150 KU, 600 KU, 5 x 600 KU
Source	Recombinant expressed in Escherichia coli	Sterile liquid
Enzyme concentration	3 KU/µL	Bioactivity: 3 KU/µL
Storage buffer	10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, pH 7.4 at 25 °C	Keep enzyme on ice

Parameter	Specification	Notes
Purity	Free of other DNA ligases, endonucleases, exonucleases, RNases and phosphatases	Per product specification

Product Contents & Storage

Cat. No.	Component	150 KU	600 KU	5 x 600 KU	Storage
T750867A	T3 DNA Ligase (3 KU/μL)	50 μL	200 μL	5 x 200 μL	-20 °C; avoid freeze-thaw
T750867B	2x Reaction Buffer	0.5 mL	2 mL	5 x 2 mL	-20 °C; avoid freeze-thaw

Shelf life: Stable for 24 months at -20 °C.

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Vector DNA and insert DNA	Clean, compatible ends	Ligation substrate
Ultrapure water	DNase/RNase-free, sterile	Reaction volume adjustment
Competent cells	Application-specific	Transformation
Purification kit	Optional, especially before electroporation	Removal of salts/PEG
Thermal incubator	25 °C capability	Ligation incubation

Preparation Before Use

1. Thaw 2x Reaction Buffer on ice and mix thoroughly. ATP is required; use fresh buffer if activity is reduced after long storage.
2. Purify ligation substrates to remove high salt, EDTA, residual phosphatase, or restriction enzymes.
3. Add T3 DNA Ligase last to the reaction.

Protocol

Recommended Reaction Setup

Reagent	Volume / Amount
2x Reaction Buffer	10 µL
Vector DNA	X µL (0.020 pmol)
Insert DNA	Y µL (0.060 pmol)
Ultrapure water	9 - X - Y µL
T3 DNA Ligase (3 KU/µL)	1 µL
Total Volume	20 µL

1. Prepare the reaction on ice using the 20 µL setup table below. A 3:1 insert:linearized vector molar ratio is recommended.
2. Mix thoroughly by pipetting and centrifuge briefly to collect liquid at the tube bottom.
3. Incubate at 25 °C or room temperature for 15-30 min.
4. Immediately place the ligation product on ice after the reaction. Transfer approximately 5 µL ligation product into 50 µL competent cells for transformation; store remaining sample at -20 °C if needed.
5. Heat inactivation is generally not recommended for downstream transformation in PEG-containing systems because it can reduce transformation efficiency.

Expected Results

Expected Outcome	Description
DNA ligation / nick repair	Covalently joined DNA or repaired duplex substrates suitable for transformation or downstream analysis

Safety & Precautions

- ATP is essential for T3 DNA Ligase. The supplied 2x Reaction Buffer contains ATP and PEG 6000.
- T3 DNA Ligase acts on double-stranded DNA or nicked duplexes with a complete DNA strand. It does not directly ligate single-stranded DNA or RNA without a template.

- The standard buffer contains PEG 6000. Use a PEG-free buffer when high NaCl is required or when PEG interferes with downstream steps.
- Heat inactivation in PEG-containing buffer may reduce transformation efficiency.
- Use ultrapure sterile DNase/RNase-free water and 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

Issue	Possible Causes	Corrective Action
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

High-salt tolerant DNA ligation, cohesive/blunt-end ligation, DNA/RNA hybrid nick repair, generation of DNA-RNA or RNA-DNA fusion nucleic acids, and DNA-templated RNA ligation 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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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.