

E. coli DNA Ligase

Cat. No.: E745500 | Pack size: 200 U / 1 KU / 5 x 1 KU | Storage: -20 °C; avoid repeated freeze-thaw

Overview

E. coli DNA Ligase is an NAD⁺ and Mg²⁺-dependent recombinant DNA ligase that catalyzes phosphodiester bond formation between adjacent 5-phosphate and 3-hydroxyl groups in double-stranded DNA.

Under standard conditions, it efficiently ligates cohesive-ended double-stranded DNA and repairs nicks in double-stranded DNA, but does not ligate blunt-ended DNA efficiently without special additives.

Key Features

- Recombinant E. coli DNA ligase supplied at 10 U/μL
- NAD⁺-dependent ligase for cohesive-end DNA ligation and nick repair
- Active from 4-37 °C; heat-inactivated at 65 °C for 20 min
- Supplied with 10x Reaction Buffer
- Free of detectable contaminating nucleases, RNases, phosphatases, and other DNA ligases

Product Specifications

Parameter	Specification	Notes
Product name	E. coli DNA Ligase	DNA ligase (ATP/NAD family); CAS No. 9015-85-4
Cat. No.	E745500	Available pack sizes: 200 U, 1 KU, 5 x 1 KU
Source	Recombinant expressed in Escherichia coli	Sterile liquid
Enzyme concentration	10 U/μL	Bioactivity: 10 U/μL
Storage buffer	10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 μg/mL BSA, 50% glycerol, pH 7.4 @ 25 °C	Keep enzyme on ice during setup

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

Product Contents & Storage

Cat. No.	Component	200 U	1 KU	5 x 1 KU	Storage
E745500A	E. coli DNA Ligase (10 U/μL)	20 μL	100 μL	5 x 100 μL	-20 °C; avoid freeze-thaw
E745500B	10x Reaction Buffer	100 μL	500 μL	5 x 500 μL	-20 °C; avoid freeze-thaw; -80 °C recommended for long-term NAD stability

Shelf life: Stable for 24 months at -20 °C. Store 10x Reaction Buffer at -80 °C for long-term NAD stability when possible.

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Cohesive-ended dsDNA substrate	Clean DNA with compatible ends	Ligation substrate
Nuclease-free water	DNase/RNase-free	Reaction volume adjustment
PCR purification or DNA gel recovery kit	User-supplied	Substrate/product cleanup when needed
Thermal incubator or water bath	16 °C and 65 °C capability	Ligation and heat inactivation
Agarose or polyacrylamide gel electrophoresis system	Application-specific	Analysis of ligation efficiency

Preparation Before Use

1. Thaw reaction buffer on ice and mix thoroughly before use.

2. Keep E. coli DNA Ligase in an ice box or on ice during reaction setup.
3. Use clean DNA substrates free of excess salt, EDTA, phenol, or ethanol.

Protocol

Recommended Reaction Setup

Reagent	Volume	Final Concentration
dsDNA	x μL	up to 0.25 $\mu\text{g}/\mu\text{L}$
10x Reaction Buffer	2 μL	1x
E. coli DNA Ligase (10 U/ μL)	1 μL	0.5 U/ μL
Nuclease-free water	17 - x μL	-
Total Volume	20 μL	-

1. Prepare the reaction mixture on ice using the 20 μL system shown below. For multiple reactions, premix all components except substrate DNA and aliquot into reaction tubes.
2. Mix gently by pipetting or brief vortexing, then briefly centrifuge to collect liquid at the tube bottom.
3. Incubate at 16 °C for 30-60 min.
4. Terminate the reaction by incubation at 65 °C for 20 min if heat inactivation is required.
5. Analyze ligation products by agarose or polyacrylamide gel electrophoresis, or purify products for downstream cloning as needed.

Expected Results

Expected Outcome	Description
Cohesive-end ligation / nick repair	Formation of phosphodiester bonds in double-stranded DNA under recommended buffer and temperature conditions

Safety & Precautions

- E. coli DNA Ligase requires NAD⁺ as a cofactor. The supplied 10x Reaction Buffer contains NAD.
- E. coli DNA Ligase has very low efficiency for blunt-end ligation; T4 DNA Ligase is recommended for blunt-end ligation.

- This enzyme acts on double-stranded DNA molecules and is not recommended for ligation of single-stranded DNA or RNA.
- For long-term buffer storage, -80 °C is recommended to extend the half-life of NAD.
- 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

Cohesive-end DNA ligation and nick repair in double-stranded DNA 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.

NORTH AMERICAN SALES, SUPPORT & GENERAL INQUIRIES Aladdin Scientific Corporation 14078 Meridian Parkway, Riverside, CA 92518, USA Phone: 1-833-552-7181 Sales: sales@aladdinsci.com Customer Service: custserv@aladdinsci.com	EU SALES, LOGISTICS & LOCALIZED SUPPORT Aladdin Biochem Deutschland GmbH Westring 2, 33142 Büren, Germany Phone: +02951 9383958 Support: support.eu@aladdinsci.com
--	---

Limitations & Disclaimer

For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, household, or other unauthorized uses.

This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals.

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.