

## *E. coli* DNA Ligase

Cat. No. DL04082H, and DL10121K

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## 1. Introduction

*E. coli* DNA Ligase is an NAD<sup>+</sup>-dependent enzyme that catalyzes the formation of phosphodiester bonds between complementary 3'-hydroxyl and 5'-phosphoryl termini of double-stranded DNA (dsDNA). The enzyme works best with cohesive dsDNA ends and is also active on nicked DNA. Blunt ends can be ligated in the presence of condensing reagents such as polyethylene glycol or Ficoll. The enzyme is not effective for the formation of DNA-RNA or RNA-RNA hybrids

*E. coli* DNA Ligase is available in a 200 Unit or 1000 Unit size at a concentration of 10 Units/ $\mu$ l. A 10X Reaction Buffer is also provided.

### Applications

- Molecular cloning of dsDNA with cohesive ends.
- Blunt-end ligation in presence of 10-15% PEG and high concentrations of monovalent cations.
- cDNA cloning of products from second-strand cDNA synthesis reactions.

## 2. Product Specifications

**Storage:** Store only at  $-20^{\circ}\text{C}$  in a freezer without a defrost cycle.

**Storage Buffer:** *E. coli* DNA Ligase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1.0 mM dithiothreitol, 0.1 mM EDTA and 0.1% Triton<sup>®</sup> X-100.

**Unit Definition:** One unit of *E. coli* DNA Ligase results in the ligation of >50% of *Hind* III-digested  $\lambda$  DNA in 30 minutes at  $16^{\circ}\text{C}$  in a total reaction volume of 20  $\mu$ l. (concentration of 5' DNA termini is 0.12  $\mu$ M [300  $\mu$ g/ml]).

**10X Reaction Buffer:** is 300 mM Tris-HCl (pH 8.0), 40 mM  $\text{MgCl}_2$ , 100 mM  $(\text{NH}_4)_2\text{SO}_4$ , 12 mM EDTA and 1 mM  $\beta$ -NAD.

**Quality Control:** *E. coli* DNA Ligase is function-tested in a 20  $\mu$ l reaction containing 30 mM Tris-HCl (pH 8.0), 4 mM  $\text{MgCl}_2$ , 10 mM  $(\text{NH}_4)_2\text{SO}_4$ , 1.2 mM EDTA, 0.1 mM  $\beta$ -NAD, 6  $\mu$ g of *Hind* III-digested  $\lambda$  DNA and varying amounts of *E. coli* DNA Ligase.

**Contaminating Activity Assays:** *E. coli* DNA Ligase is free of detectable RNase, endo- and exonuclease activities.

### 3. Related Products

The following products are also available:

- APex™ Heat-Labile Phosphatase
- T4 Polynucleotide Kinase
- Fast-Link™ DNA Ligation Kits
- Fast-Link™ DNA Ligation and Screening Kits
- Colony Fast-Screen™ Kits
- Ampligase™ Thermostable DNA Ligase
- T4 DNA Ligase
- End-It™ DNA End-Repair Kit

### 4. Reference

Sambrook, J. *et al.*, (2001) in: *Molecular Cloning: A Laboratory Manual* (3rd ed.), Cold Spring Harbor Laboratory Press, New York.

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