

# *E. coli* DNA Ligase



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M0205S 075131015101

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200 units Lot: 0751310 Exp: 10/15

10,000 U/ml Store at -20°C

**Description:** Catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA containing cohesive ends.

**Source:** Purified from *E. coli* strain 594 (su<sup>-</sup>) carrying the prophage  $\lambda$ gt4 *lop*11 *lig*<sup>+</sup>*Sam* 7 (1) by the procedure of Panasenko et al. (2)

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### Applications:

- Okayama and Berg cDNA cloning (3)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200  $\mu$ g/ml BSA and 50% glycerol.

### Reagents Supplied with Enzyme:

10X *E. coli* DNA Ligase Buffer.

**Reaction Conditions:** Incubate DNA at a final concentration of 0.12  $\mu$ M in 20–50  $\mu$ l of 1X *E. coli* DNA Ligase Buffer at 16°C overnight.

### 1X *E. coli* DNA Ligase Buffer:

30 mM Tris-HCl  
4 mM MgCl<sub>2</sub>  
1 mM DTT  
26  $\mu$ M NAD<sup>+</sup>  
50  $\mu$ g/ml BSA  
pH 8.0 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of  $\lambda$  DNA (5' DNA termini concentration of 0.12  $\mu$ M, 300  $\mu$ g/ml) in a total reaction volume of 20  $\mu$ l in 30 minutes at 16°C.

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**Unit Assay Conditions:** 30 mM Tris-HCl (pH 8.0), 4 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 26  $\mu$ M NAD<sup>+</sup>, 50  $\mu$ g/ml bovine serum albumin and Hind III fragments of  $\lambda$  DNA (300  $\mu$ g/ml).

**Specific Activity:** ~ 6,000 units/mg.

**Heat Inactivation:** 65°C for 20 minutes.

### Quality Control Assays

**Exonuclease Activity:** Incubation of 20 units of enzyme for 4 hours at 37°C in 50  $\mu$ l assay buffer containing 1  $\mu$ g sonicated *E. coli* <sup>3</sup>H DNA (10<sup>5</sup> cpm/ $\mu$ g) resulted in < 0.1% acid soluble counts.

**16 Hour-Incubation:** Incubation of 20 units of *E. coli* DNA Ligase with 1  $\mu$ g of  $\lambda$  DNA (HindIII digest) for 16 hours in 50  $\mu$ l of 1X NEBuffer 3 at 37°C resulted in a normal and sharp banding pattern on agarose gels.

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**Notes on Use:** Requires NAD<sup>+</sup> (nicotinamide adenine dinucleotide) as a cofactor, in contrast to other ligases which use rATP.

Ligation of blunt-ended fragments is extremely inefficient. For ligation of blunt-ended fragments use T4 DNA Ligase.

Does not ligate RNA to DNA (4).

This enzyme ligates only DNA fragments with cohesive termini.

### References:

1. Panasenko, S. M. et al. (1977) *Science* 196, 188–189.
2. Panasenko, S. M. et al. (1978) *J. Biol. Chem.* 253, 4590–4592.
3. Okayama, H. and Berg, P. (1982) *Mol. Cell. Biol.* 2, 161–170.
4. Higgins, N. P. and Cozzarelli, N. R. (1979) *Methods Enzymol.* 68, 50–71.

CERTIFICATE OF ANALYSIS

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