

E. coli DNA Ligase



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200 units Lot: 0521304 Exp: 4/15

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⁻) carrying the prophage λ gt4 *lop*11 *lig*⁺*Sam* 7 (1) by the procedure of Panasenکو 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 μ 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 μ M in 20–50 μ l of 1X *E. coli* DNA Ligase Buffer at 16°C overnight.

1X *E. coli* DNA Ligase Buffer:

30 mM Tris-HCl
4 mM MgCl₂
1 mM DTT
26 μ M NAD⁺
50 μ 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 λ DNA (5' DNA termini concentration of 0.12 μ M, 300 μ g/ml) in a total reaction volume of 20 μ l in 30 minutes at 16°C.

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Unit Assay Conditions: 30 mM Tris-HCl (pH 8.0), 4 mM MgCl₂, 1 mM dithiothreitol, 26 μ M NAD⁺, 50 μ g/ml bovine serum albumin and Hind III fragments of λ DNA (300 μ 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 μ l assay buffer containing 1 μ g sonicated *E. coli* ³H DNA (10⁵ cpm/ μ g) resulted in < 0.1% acid soluble counts.

16 Hour-Incubation: Incubation of 20 units of *E. coli* DNA Ligase with 1 μ g of λ DNA (HindIII digest) for 16 hours in 50 μ 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⁺ (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. Panasenکو, S. M. et al. (1977) *Science* 196, 188–189.
2. Panasenکو, 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.

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