E. coli DNA Ligase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0205S 007150117011

M0205S

200 units **Lot: 0751501** **Exp: 1/17**
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 containing a cloned *E. coli* DNA Ligase gene.

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 µg/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 µM in 20–50 µl of 1X *E. coli* DNA Ligase Buffer at 16°C overnight.

1X *E. coli* DNA Ligase Buffer:
30 mM Tris-HCl
4 mM $MgCl_2$
1 mM DTT
26 µM NAD^+
50 µg/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 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16°C.

Unit Assay Conditions: 30 mM Tris-HCl (pH 8.0), 4 mM $MgCl_2$, 1 mM dithiothreitol, 26 µM NAD^+, 50 µg/ml bovine serum albumin and Hind III fragments of λ DNA (300 µg/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 µl assay buffer containing 1 µg sonicated *E. coli* ^3H DNA (10^5 cpm/µg) resulted in < 0.1% acid soluble counts.

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

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. 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.

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CERTIFICATE OF ANALYSIS