



DNA Ligase

(*Escherichia coli*)

Cat. No.	size
E1065-01	500 units
E1065-02	2 500 units

Unit Definition:

One unit is defined as the amount of enzyme required to yield 50% ligation of HindIII fragments of lambda DNA. Incubation is at 16°C in 20 µl of assay mixture with a DNA terminus concentration of 0.02 µM (50 µg/ml).

Storage Conditions:

Store at -20°C.

References:

1. Okayama, H. and Berg, P. (1982) *Mol. Cell. Biol.* 2, 161-170.
2. Gubler, U and Hoffman, B.J. (1983) *Gene* 25, 263-269.

DNA Ligase (*E.coli*) catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl cohesive termini in duplex DNA.

Description:

- Catalyzes the formation of a phosphodiester bond between duplex DNA fragments with cohesive ends.
- Condensation of the 5'-phosphoryl group with an adjacent 3'-hydroxyl group is coupled with the hydrolysis of NAD⁺.
- Suitable for high-efficiency cloning of full-length cDNA (1, 2).

Ligation Assay Conditions:

30 mM Tris-HCl (pH 8.0 at 22°C), 4 mM MgCl₂, 1 mM dithiothreitol, 0.026 mM NAD⁺, 50 µg/ml bovine serum albumin and HindIII fragments of lambda DNA. Incubation was carried out at 16°C for 30 min.

1 x Reaction Buffer:

30 mM Tris-HCl (pH 8.0 at 22°C), 1 mM dithiothreitol, 4 mM MgCl₂, 26 µM NAD⁺, 25 µg/ml bovine serum albumin.
Optimal ligation occurs at 16°C.

Storage Buffer:

10 mM Tris-HCl (pH 7.4 at 22°C), 50 mM KCl, 0.1 mM EDTA, 10 mM ammonium sulfate, 1 mM dithiothreitol and 50% (v/v) glycerol.

Quality Control:

All preparations are tested for contaminating endonuclease and exonuclease activities, along with functional testing in the ligation reaction.