9°N™ DNA Ligase

1-800-632-7799
info@neb.com
www.neb.com

M0238S 003140516051

M0238S

2,500 units	**40,000 U/ml**	**Lot: 0031405**
RECOMBINANT	**Store at –20°C**	**Exp: 5/16**

Description: 9°N DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3´ hydroxyl termini of two adjacent oligonucleotides which are hybridized to a complementary target DNA. 9°N DNA Ligase is active at elevated temperatures (45°C–90°C).

Source: Purified from an *E. coli* strain containing the cloned ligase gene from the extremely thermophilic marine archaea *Thermococcus* sp.(strain 9°N). The arcahea was isolated from a submarine thermal vent, at a depth of 2,500 meters, 9° north of the equator at the East Pacific Rise (1).

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 10 mM ammonium sulfate, 200 µg/ml BSA and 50% glycerol.

Applications:

- Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction (2,4).
- Mutagenesis by incorporation of a phosphorylated oligonucleotide during PCR amplification (5).

Reagents Supplied with Enzyme:
10X 9°N DNA Ligase Reaction Buffer:

Reaction Conditions: Incubate DNA and enzyme in 1X 9°N DNA Ligase Reaction Buffer at 45°C for 15 minutes or in a thermocycler with a program suited to the reaction described by Barany (1991) Genetic Disease Detection and DNA Amplification Using Cloned Thermostable Ligase. *Proc. Natl. Acad. Sci. USA* 88, 189–193. The reaction is stopped with a mixture of 50% glycerol, 50 mM EDTA, bromphenol blue.

1X 9°N DNA Ligase Reaction Buffer:
10 mM Tris-HCl
600 µM ATP
2.5 mM dithiothreitol
2.5 mM $MgCl_2$
0.1% Triton X-100
(pH 7.5 @ 25°C)

Unit Definition: (Cohesive End Unit)
One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C.

A cohesive end unit is equivalent to the nick-closing unit (1).

Unit Assay Conditions: 1X 9°N DNA Ligase Reaction Buffer and 20 µg/ml BstEII-digested λ DNA in a 50 µl reaction. After incubation at 45°C for 15 minutes, the reaction is terminated by addition of stop dye (50% glycerol, 50 mM EDTA and bromophenol blue), heated at 70°C for 10 minutes and then loaded on a 0.7% agarose gel. Due to the presence of ligase, the cos ends of BstEII-digested λ DNA will stay together after 70°C heat treatment.

Heat Inactivation: No

Quality Control Assays

Exonuclease Activity: Incubation of 1,200 units for 4 hours at 37°C in 50 µl of thermostable ligase buffer containing 1 µg sonicated ^3H (10^5 cpm/µg) gave < 0.1% acid soluble counts.

Nuclease Activity: Incubation of HindIII fragments of λ DNA with 80 units of enzyme for 16 hours at 37°C in the recommended assay buffer without NAD does not alter the λ HindIII banding pattern on agarose gels.

Endonuclease Activity: Incubation of 1,500 units of enzyme for 4 hours at 37°C in 50 µl of assay buffer without NAD and 1 µg ϕX174 RF I DNA gave < 10% conversion to RF II

Note: 9°N DNA Ligase is not a substitute for T4 DNA Ligase. The cohesive end unit is equivalent to the nick-closing unit of Barany et al.

References:

1. *Thermococcus* sp. (strain 9°N-7) isolated by Dr. Holger Jannasch, Woods Hole Oceanographic Institute, 1991.

(see other side)

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2. Barany, F. (1991) *Proc. Natl. Acad. Sci. USA* 88, 189-193.
3. Takahashi, M. et al. (1984) *J. Biol. Chem.* 259, 10041–10047.
4. Barany, F. (1991) *The Ligase Chain Reaction in a PCR World* (pp. 5–16). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
5. Michael, S.F. (1994) *Biotechniques* 16, 411–412.

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2. Barany, F. (1991) *Proc. Natl. Acad. Sci. USA* 88, 189-193.
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