Taq DNA Ligase

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

M0208S 0071209140911

M0208S

RR NO

2,000 units **40,000 U/ml** **Lot: 0071209**
RECOMBINANT **Store at –20°C** **Exp: 9/14**

Description: *Taq* 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. The ligation will occur only if the oligonucleotides are perfectly paired to the complementary target DNA and have no gaps between them; therefore, a single-base substitution can be detected. *Taq* DNA Ligase is active at elevated temperatures (45–65°C) (1,2).

Source: Purified from an *E. coli* strain containing the cloned ligase gene from *Thermus aquaticus* HB8 (1)

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.

Applications:

- Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction (1,3).
- Mutagenesis by incorporation of a phosphorylated oligonucleotide during PCR amplification (4).

Reagents Supplied with Enzyme: 10X *Taq* DNA Ligase Reaction Buffer and 5 µg control DNA (BstEII-digested λ DNA).

Reaction Conditions: Incubate DNA and enzyme in 1X *Taq* DNA Ligase 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 *Taq* DNA Ligase Reaction Buffer:
20 mM Tris-HCl
25 mM potassium acetate
10 mM magnesium acetate
10 mM dithiothreitol
1 mM NAD
0.1% Triton X-100
(pH 7.6 @ 25°C)

Requires NAD^+ as a cofactor. NAD^+ is supplied in the 10X *Taq* DNA Ligase Reaction Buffer; the buffer should be stored at –70°C to extend the half life of the NAD^+ cofactor.

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.

Unit Assay Conditions: 1X *Taq* DNA Ligase Reaction Buffer and DNA (20 µg/ml). 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 DNA (10^5 cpm/µg) gave < 0.1% acid soluble counts.

Nuclease Activity: Incubation of 4,000 units for 16 hours at 37°C in the recommended assay buffer plus NAD with SmaI-SalI fragments of λ DNA does not alter the expected banding pattern on agarose gels. 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.

(see other side)

CERTIFICATE OF ANALYSIS

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References:

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

Taq DNA Ligase: Notice to Purchaser: This product is designed to ligate DNA fragments at temperatures requiring a thermoactive and thermostable enzyme. The seller is aware that the product may be used in the Ligase Chain Reaction™ (LCR™) process covered by one of more claims of a pending patent application or issued patent assigned to Cornell Research Foundation Inc., or Cornell Research Foundation, Inc. and the California Institute of Technology. LCR™ license inquires should be directed to Cornell Research Foundation, Inc.

References:

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