Taq DNA Ligase

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 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, bromophenol 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 λ DNA (106 cpm/µg) gave < 0.1% acid soluble counts.

Non-Specific DNase Activity (16 hours): A 50 µl reaction in NEBuffer 4 containing 1 µg of λ HindIII DNA and 80 units of Taq DNA Ligase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nucleic acid degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of 1,500 units of enzyme for 4 hours at 37°C in 50 µl assay buffer without NAD and 1 µg eX174 RF I DNA gave < 10% conversion to RF II.

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References:


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 or more claims of a pending patent application or issued patent assigned to Cornell Research Foundation Inc., Cornell Research Foundation, Inc. and the California Institute of Technology. LCR™ license inquiries should be directed to Cornell Research Foundation, Inc.