Taq DNA Ligase

Catalogue number: MB42601, 2000 U

Description

Taq DNA Ligase is a NAD^+ dependent thermostable DNA ligase that catalyses the formation of a phosphodiester bond between juxtaposed 5′ phosphate and 3′ hydroxyl termini of two adjacent nucleotides in the context of a double-stranded DNA molecule. The ligation will occur only if the adjacent nucleotides are perfectly paired and have no gaps between them, thus allowing the use of this enzyme as a tool to detect single-base substitutions. As expected from its origin from a thermophilic host, Taq DNA Ligase is active at elevated temperatures (45–65°C). Due to critical differences in substrate specificity, Taq DNA Ligase is not a substitute for T4 DNA Ligase and it is not suitable for cloning applications or adapter ligation/NGS library prep. Taq DNA Ligase is primarily used in molecular diagnostic applications and for direct cloning applications.

Storage conditions

Taq DNA Ligase should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit of enzyme activity is defined as the amount of enzyme required to ligate the 12-base pair cohesive ends of 1 µg of BstEII-digested λDNA in a total reaction volume of 50 µl in 15 minutes at 45°C.

Enzyme concentration: 40 U/µL

Inactivation

Taq DNA Ligase is highly resistant to heat inactivation. Thus, alternative protocols should be considered when requiring removing the enzyme from reactions, such as DNA silica column purification or phenol/chloroform extraction.

System components and Reaction conditions

Taq DNA Ligase is provided with a dedicated and highly optimized NZYTech reaction buffer containing NAD^+ and displays an optimum temperature of 55 °C.

Standard protocol

The following standard protocol serves as a general guideline to perform oligonucleotide probe ligation with Taq DNA Ligase (***note**: the enzyme is not active in supercoiled dsDNA*). The optimal ligation incubation temperature for a given set of probes is typically within 5°C of the Tm of the probes annealing region and must be determined empirically for the best balance of activity and fidelity in your application. LDR and LCR reactions with Taq DNA Ligase should be performed between 45-65°C.

1. Prepare the following 50 µL reaction:

Component	Volume
Substrate DNA	≤ 1 µg
Taq Ligase reaction buffer (10x)	5 µL
Taq DNA Ligase	1 µL (40 U)
Nuclease-free H_2O (Cat. No. MB11101)	up to 50 µL

***Note:** It may be required to titrate the enzyme or test different incubation periods for more effective results.*

2. Gently mix and pulse.
3. Incubate at 55 °C for 15 minutes.

Quality Control Assays

Purity

Taq DNA Ligase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYTech, Cat. No. MB15201).

Nucleases assays

To test for DNAse contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 40 U of Taq DNA Ligase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 40 U of Taq DNA Ligase for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

Taq DNA Ligase is functionally tested by its capacity to ligate the 12-base pair cohesive ends of 1 µg of BstEII-digested λDNA in a total reaction volume of 50 µl in 15 minutes at 45°C.

V2101

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases contamination	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
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