DNA Ligase (*E. coli*)

Catalogue number: MB42401, 200 U

Description

DNA Ligase (*E. coli*) is a typical DNA ligase that catalyses the formation of a phosphodiester bond between the 5´-phosphate and the 3´-hydroxyl of two adjacent DNA strands in duplex DNA. Thus, the enzyme is particularly effective to repair nicks in dsDNA although it can be used to ligate two DNA fragments with compatible cohesive ends. DNA Ligase (*E. coli*) is not significantly active on blunt-ended substrates. DNA Ligase (*E. coli*) uses NAD as a cofactor and can be heat-inactivated. The enzyme is active at a range of temperatures (4 °C – 37 °C).

Storage conditions

DNA Ligase (*E. coli*) 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 give a 50% ligation of a *Sal*I-digested DNA fragment in a total reaction volume of 20 µL in 30 minutes at 16 °C in 1x DNA Ligase (*E. coli*) Reaction Buffer.

Enzyme concentration: 10 U/µL

Inactivation

DNA Ligase (*E. coli*) is heat inactivated at 65°C for 20 min.

System components

DNA Ligase (*E. coli*) is provided with a dedicated and highly optimized NZYtech reaction buffer supplemented with NAD.

Standard protocol

Optimum temperature for ligation is 16 °C, although enzyme performs well at temperatures ranging from 4 °C – 37 °C.

The following standard protocol serves as a general guideline to ligate cohesively ended DNA fragments with DNA Ligase (*E. coli*) (the enzyme does not perform well in the ligation of blunt-ended substrates). Preferably the enzyme should be added last.

1. Prepare the following 20 µL reaction:

Component	Volume
Substrate DNA	≤ 1 µg
DNA Ligase (*E. coli*) reaction buffer (10x)	2 µL
DNA Ligase (*E. coli*)	1 µL (10 U)
Nuclease-free H_2O (Cat. No. MB11101)	up to 20 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 16 °C for 30 minutes.
4. If required, heat inactivate by incubating at 65°C for 20 minutes.
5. To obtain a highly pure product, perform a column purification step using NZYGelpure kit (Cat. No. MB011).

Quality Control Assays

Purity

DNA Ligase (*E. coli*) 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 10 U of DNA Ligase (*E. coli*) for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of DNA Ligase (*E. coli*) 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

DNA Ligase (*E. coli*) is assayed in a re-ligation protocol using as substrate a *Sal*I-digested DNA plasmid. The ligation product is then transformed into NZY5α competent cells and the ligation efficiency is determined by counting transformed bacterial colonies.

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