T7 DNA Ligase

Catalogue number: MB42501, 100,000 U

Description

T7 DNA Ligase is a typical DNA ligase that catalyses the formation of a phosphodiester bond between adjacent 5´ phosphate and 3´ hydroxyl groups of duplex DNA molecules. Cohesive end ligation and nick sealing are efficiently catalysed by T7 DNA Ligase, although the enzyme does not perform blunt end ligations. Thus, the enzyme discriminates between cohesive (active) and blunt (not active) fragment DNA ligations, as it only performs sticky end ligations. T7 DNA Ligase requires ATP for activity.

Storage conditions

T7 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 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 1× DNA Ligase Reaction Buffer.

Enzyme concentration: 3000 U/ µL

Inactivation

T7 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

T7 DNA Ligase is provided with a dedicated and highly optimized NZYtech reaction buffer containing ATP.

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 T7 DNA Ligase (the enzyme does not perform 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
T7 DNA Ligase reaction buffer (4x)	5 µL
T7 DNA Ligase	1 µL (3000 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. To obtain a highly pure product, perform a column purification step using NZYGelpure kit (Cat. No. MB011). Best results may be achieved by separating cleaved DNA through agarose gel electrophoresis prior to DNA clean-up.

Quality Control Assays

Purity

T7 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 10 U of T7 DNA Ligase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of T7 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

T7 DNA Ligase 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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