

T4 DNA Ligase

Catalogue number:

MB00703, 500 U MB00704, 2500 U

Description

T4 DNA Ligase is an ultrapure recombinant enzyme purified from *Escherichia coli* and supplied with an optimized 4× Reaction Buffer. T4 DNA ligase catalyses the formation of a phosphodiester bond between juxtaposed 5'-phosphoryl and 3'-hydroxyl termini in duplex DNA. It repairs single-strand nicks in duplex DNA and will join both blunt and cohesive-end restriction fragments of duplex DNA or RNA. The enzyme requires ATP as cofactor.

Storage temperature

T4 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 catalyses the exchange of 1 nmol of radiolabelled phosphate from pyrophosphate into Norit-absorbable material in 20 min at 37 $^\circ C$ under standard assay conditions.

Enzyme concentration: 5 U/µL

Inactivation

T4 DNA ligase is heat inactivated at 65 °C for 10 min.

System components

T4 DNA Ligase is provided with a dedicated highly optimized NZYtech reaction buffer, which contains ATP that is critical for this enzyme. Repeated freeze-thaw cycles will affect the stability of ATP, so we recommend making 10-20 μ L aliquots of the reaction buffer and store at -20 °C. Vortex the reaction buffer solution thoroughly after thawing and prior to use.

Ligation Protocol

The enzyme performs well at temperatures ranging from 16 °C to 25 °C. The optimal temperature for a ligation reaction is a balance between the enzyme's optimal temperature and the temperature required to ensure annealing of the DNA fragment ends, which can vary with the length and base composition of the terminal sequences.

We recommend using a 1:3-10 molar ratio of vector:insert. To calculate optimal amounts of insert DNA in ligation reaction, see below:

<u>ng of vector × kb size of insert</u> × molar ratio of <u>insert</u> = ng of insert kb size of vector vector **Example:** If using 50 ng of a vector plasmid with 3 kb, for a 1:10 molar ratio of vector:insert then you will require the following amount of a 500 bp insert:

1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components (volumes for a 20 μ L reaction):

Component	Volume
4× Reaction buffer (provided)	5 μL
Vector DNA (20-50 ng)	×μL
Insert DNA (3-10 molar excess)	yμL
T4 DNA Ligase (5 U/μL)	1 μL
Nuclease-free water	up to 20 μL

2. Mix and centrifuge briefly to bring the contents to the bottom of the tube.

- 3. Incubate at 16-20 °C for 16 hours.
- 4. Use the ligation reaction to transform NZYtech competent cells.

Important notes

- 4× reaction buffer is highly viscous, so it is recommended special care while pipetting. 4× reaction buffer should be thoroughly vortexed before pipetting.
- It is extremely important not to change the ratio between the volume of T4 DNA Ligase and the reaction final volume to prevent decrease in efficiency of cloning reactions.
- For blunt-end ligations, use higher quantities of both vector and insert DNA.
- For sticky (cohesive)-end ligations, we recommend to heat both vector and insert DNA prior to the ligation.
- If the ligation mixture will be used for electroporation, a DNA purification step is recommended before the transformation. Use a spin column purification method (NZYGelpure, Cat. No. MB011) or chloroform extraction.

Quality control assays

Purity

Recombinant T4 DNA Ligase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe (NZYtech, Cat. No. MB152) staining.

Nuclease assays

0.2-0.3 μ g of pNZY28 plasmid DNA are incubated with 5 U of T4 DNA Ligase in 1× Reaction buffer for 14-16 hours at 37 °C. Following incubation, the DNA is visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the DNA.

Functional assay

Linearized pNZY28 plasmid (leaving either blunt-end or cohesive ends) is re-ligated with 5 U of T4 DNA Ligase. The DNA is then transformed into NZY5 α competent cells that are plated on ampicillin plates. The re-ligation efficiency is determined by counting transformed bacterial colonies.

Related products

Product name	Cat. No.
ΝΖΥ5α	MB004
NZYStar	MB005
Speedy Ligase	MB130
NZYGelpure	MB011

V2401

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