T4 DNA Ligase

Catalogue number	Presentation
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.

Shipping & Storage Conditions

This product is shipped in blue to dry ice. Upon receipt, store all components at -80 °C to -15 °C. These meticulous storage procedures ensure that T4 DNA Ligase delivers consistent and reliable results across its lifespan and usage. All components are formulated to be ready to use. The kit will remain stable till the expiry date if stored as specified.

Components

	MB00703 (500 U)		MB00704 (2500 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
T4 DNA Ligase	1	100 µL	5	100 µL
4x Reaction Buffer for T4 DNA Ligase	1	600 µL	2	600 µL

Specifications

Unit Definition: One unit catalyses the exchange of 1 nmol of radiolabelled phosphate from pyrophosphate into Norit-absorbable material in 20 min at 37 °C under standard assay conditions.

Enzyme concentration: 5 U/µL.

Inhibition & Inactivation: T4 DNA ligase is heat inactivated at 65 °C for 10 min.

Standard Protocol

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 µL 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:

$$\frac{ng\ of\ vector \times kb\ size\ of\ insert}{kb\ size\ of\ vector} \times molar\ ratio\ of\ \frac{insert}{vector} = ng\ of\ insert$$

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:

$$\frac{50 \times 0.5}{3} \times 10 = 83 \text{ ng}$$

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

Component	Volume
4× Reaction buffer (provided)	5 µL
Vector DNA (20-50 ng)	x µL
Insert DNA (3-10 molar excess)	y µL
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.

Technical 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

Purity

Recombinant T4 DNA Ligase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Nuclease assays

0.2-0.3 µg 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 NZY5α competent cells that are plated on ampicillin plates. The re-ligation efficiency is determined by counting transformed bacterial colonies.