# T4 DNA Ligase

**Catalogue number:** MB00703, 500 U
MB00704, 2500 U

## Description

T4 DNA Ligase is an ultrapure recombinant enzyme purified from *Escherichia coli* supplied with an optimized 10× 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 non-frost free freezer.

## Storage buffer

20 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA and 50% (v/v) glycerol.

## Unit definition

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

**Enzyme concentration:** 5 U/µL

**Reaction buffer (10×):** 660 mM Tris-HCl, pH 7.6, 66 mM $MgCl_2$, 100 mM DTT, 660 µM ATP. Vortex the reaction buffer solution thoroughly after thawing and prior to use. Repeated freeze-thaw cycles will affect the stability of ATP. We recommend making 10-20 µL aliquots of the buffer and storage at -20 °C.

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

## Related products:

| Product name | Cat. No. |
|---|---|
| NZY5α | MB004 |
| NZYStar | MB005 |
| Speedy Ligase | MB130 |
| NZYGelpure | MB011 |

## Ligation Protocol

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:

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

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

| Component | Volume |
|---|---|
| 10× Reaction buffer (provided) | 2 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.

## Important notes

- It is extremely important not to change the ratio of T4 DNA Ligase volume: 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, MB011) or chloroform extraction.

## Quality control assays

### 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 units of T4 DNA Ligase. The DNA is then transformed into NZY5α cells that are plated on ampicillin plates. The re-ligation efficiency is determined by counting transformed bacterial colonies.

Revised 12/12

## Certificate of Analysis

| Test | Result |
|---|---|
| Enzyme purity | Pass |
| Nucleases assays | Pass |
| Functional assay | Pass |

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