Product information

T4 DNA LIGASE

Catalog #:	B1125/B1122
Concentration:	5U/ul
Storage:	-20°C

Enzyme Description:

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. The enzyme repairs single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids, joins DNA fragments with either cohesive or blunt termini. The T4 DNA Ligase requires ATP as a cofactor.

Source:

E.coli cells with a cloned gene 30 from bacteriophage T4.

Molecular Weight:

55.3 kDa monomer.

Storage Buffer:

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% (v/v) glycerol.

10X Reaction Buffer:

400 mM Tris-HCl, 100 mM MgCl2, 100 mM DTT, 5 mM ATP (pH 7.8 at 25°C).

50% PEG Solution:

50% (w/v) polyethylene glycol 4000.

Unit Definition:

One Weiss unit of the enzyme catalyzes the conversion of 1 nmol of [32PPi] into Norit-adsorbable form in 20 min at 37°C (4). One Weiss unit is equivalent to approximately 200 cohesive end ligation units (CEU)*.

Enzyme activity is assayed in the following mixture:
66 mM Tris-HCl (pH 7.6), 6.6 mM MgCl2, 0.066 mM ATP, 10 mM DTT, 3.3 µM [32PPi].

*: One CEU is defined as the amount of enzyme required to give 50% ligation.

Application:

Cloning of restriction enzyme generated DNA fragments.
Cloning of PCR products.
Joining of double-stranded oligonucleotide linkers or adaptors to DNA.
Site-directed mutagenesis.
Amplified fragment length polymorphism (AFLP).
Ligase-mediated RNA detection.
Nick repair in duplex DNA, RNA or DNA/RNA hybrids.
Self-circularization of linear DNA.

Inhibition and Inactivation:

T4 DNA Ligase is strongly inhibited by NaCl or KCl at concentrations higher than 200mM.
Inactivated by heating at 65°C for 10 min or at 70°C for 5 min.

Protocols - DNA INSERT LIGATION INTO VECTOR DNA:

Sticky-end ligation:

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 5:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2µl
T4 DNA Ligase	1 U to 5 U
Water, nuclease-free	to 20 µl
Total volume	20 µl

2. Incubate 10 min at 22°C.

3. Use 1µl -5 µl of the mixture for transformation of 50 µl of competent cells.

Blunt-end ligation:

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 5:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2µl
50% PEG 4000 Solution	2µl
T4 DNA Ligase	5 U
Water, nuclease-free	to 20 µl
Total volume	20 µl

2. Incubate for 1 hour at 22°C.

3. Use 1µl -5 µl of the mixture for transformation of 50 µl of competent cells.

Note:

The transformation efficiency may be improved by:

– heat inactivation of T4 DNA ligase at 65°C for 10 min or at 70°C for 5 min,

– Increase or extend ligation to 1 hour 22°C.

– Keep ratio of ligation mixture: competent cells less than 10%. In other words, do not exceed usage of 5ul of ligation mixture in a tube containing 50ul of competent cells.

