FOR RESEARCH USE ONLY!

T4 DNA ligase

04/21

(Catalog # M1514-4000; 4000 U); Store at -20 °C)

I. Introduction:

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme joins blunt-end and cohesive-end termini as well as repairs single-stranded nicks in duplex DNA and some DNA/RNA hybrids. T4 DNA Ligase seals nicks for these DNA substrates. T4 DNA Ligase can be used for cloning restriction fragments and joining linkers and adapters to blunt-ended DNA.

II. Applications:

- Ligate linkers and adapters to blunt-ended DNA
- Cloning restriction fragments
- For blunt-end and cohesive-end DNA ligation

III. Key Features:

- RNase, nuclease and exonuclease free
- No residual host genomic DNA detected by PCR

IV. Concentration: 400 U/µl

V. Form: Liquid

VI. Purity: ≥ 95% by SDS-PAGE

VII. Product Source: *E.coli* strain that carries the T4 DNA ligase gene

VIII. Contents:

Components	Part Number	Volume
T4 DNA Ligase (400 U/µl)	M1514-4000	10 µl

IX. Enzyme Storage Buffer:

In 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol

X. Enzyme Unit Definition:

One unit is defined as the amount of enzyme required to give 50% ligase of HindIII digestion fragments ligate and 50% of HindIII digestion fragments of λ DNA (5´ DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl over 30 min at 16 °C in 1X T4 DNA Ligase Reaction Buffer.

XI. Storage Conditions:

Store all components at -20 °C.

XII. Heat Inactivation:

Incubate at 65 °C for 20 min.

XIII. Protocol:

1. Set up the following reaction on ice.

Component	Volume
10X T4 DNA Ligase Reaction Buffer*	2 µl
Vector DNA (4 kb)	50 ng (0.02 pmol)
Insert DNA (1 kb)**	37.5 ng (0.06 pmol)
Nuclease-free Water	Upto 19 µl
T4 DNA Ligase***	1 µl

*10X T4 DNA Ligase Reaction Buffer (not provided, 1X T4 DNA Ligase Reaction Buffer: 50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH 7.5) should be thawed and resuspended at room temperature
**Insert DNA (1 kb): A ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes
***T4 DNA Ligase should be added in the end

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. For cohesive (sticky) ends, incubate at 16 °C overnight or room temperature (RT) for 10 min.
4. For blunt-ends or single-base overhangs, incubate at 16 °C overnight or RT for 2 h.
5. Heat inactivate at 65 °C for 10 min.
6. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

XIV. Related Products:

BioVision Product Name	Cat. No.	Sizes
T4 RNA Ligase 2 (Truncated)	M1220	100 µl
T4 RNA Ligase 2 (dsRNA Ligase)	M1219	100 µl
Link-FAST™ 5 Minutes DNA Ligation Kit	K902	50 ligations

T4 DNA Ligase Buffer	9102	1 ml
T4 DNA Polymerase	M1211	100 µl

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