



## T4 DNA Ligase

**Cat. No.:** MO5451

**Store at** -20°C

**Concentration:** 350u/μl, 5000U

**Supplied with:** 35μl of 10X Reaction Buffer

### Description

Purified from an *E.coli* strain carrying a plasmid with the cloned gene of phage T4 encoding this enzyme. T4 DNA Ligase catalyzes the formation of a phosphodiester bonds between 5' phosphate and 3' hydroxyl termini in duplex DNA/RNA. This enzyme can join-blunt end and cohesive-end termini, repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

### Definition of Activity Unit

One unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of lambda DNA in 30 minutes at 16°C in the reaction mixture of 20μl.

### Activity assay

40mM Tris-HCL(pH7.5), 10mM MgCl<sub>2</sub>, 10mM DTT, 1mM ATP. Optimal temperature is 16°C.

### Application

Cloning of restriction fragments, joining linkers and adapters to blunt-ended DNA, gene (gene fragments) synthesis.

### Ligation

For most cohesive-end ligations, at least 60 minutes incubation at room temperature is sufficient. Incubations at 16°C for 4-16 hours are routinely used for the majority of applications.

Ligation of blunt-ends and single-base pair overhang fragments requires more enzyme to achieve the same extent of ligation as cohesive-end DNA fragments. Ligation can be enhanced by addition of PEG or by reducing the rATP concentration.

ATP is an essential cofactor for the reaction.

### Storage Conditions

50mM KCl, 50mM Tris-HCl (pH7.5), 0.1mM EDTA, 1mM DTT, 50% glycerol at -20°C.

### QC

tested for the absence of endo-exodeoxyribonucleases, ribonucleases.

### Inactivation Conditions

65 °C for 15 minutes or boiling for 2 minutes

SinaClon BioScience

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