T4 DNA Ligase

Cat No. MB100-0100
Size: 100 units
Conc. 1 U/µl
Store at -20°C (in a non frost–free freezer)

Description

The T4 DNA Ligase catalyzes the formation of phosphodiester bonds between 3'-OH termini and 5'-P termini. Its MW is about 62,000, and its optimal reaction pH is 7.6. It requires Mg^{2+} and ATP as cofactors. This enzyme can ligate both cohesive end pair and blunt end pair.

Unit Definition

One unit is the amount of enzyme that ligates more than 90% of 6 µg of λDNA-HindIII fragments in a 20 µl mixture in 30 minutes at 16°C.
One unit of this enzyme corresponds to 0.008 Weiss units by the ATP-PPi exchange reaction in 66 mM Tris-HCl (pH 7.6) that contains 6.6 mM $MgCl_2$, 10 mM DTT, 66 µM ATP, and 3.3 µM [32P]-$Na_4P_2O_7$.

Components

T4 DNA Ligase
10X T4 DNA Ligase Buffer

Applications

1. Insertion of the DNA fragment into a vector.
2. Linker ligation with DNA fragment.

Protocols

T4 DNA Ligase	10× T4 DNA Ligase Buffer
10 mM Tris-HCl (pH7.5)	660 mM Tris-HCl, pH7.6
50 mM KCl	66 mM $MgCl_2$
1 mM DTT	100 mM DTT
0.1 mM EDTA	1 mM ATP
50% Glycerol	

Source

Escherichia coli carrying the plasmids that enable highy expression of T4 DNA ligase gene.

Purity

Nuclease activity is not detected in any of the following cases, as judged from the intact gel electrophoresis pattern:
1. After incubation of 1 µg of λDNA-Hind III fragments with 2,000 units of this enzyme for 16 hours at 37°C.
2. After incubation of 1 µg of supercoiled pBR322 DNA with 2, 000 units of this enzyme for 16 hours at 37°C.
3. After incubation of 1 µg of 16s, 23s-rRNA with 2,000 units of this enzyme for 16 hours at 37°C.

Note : The reaction rate of ligation varies with the terminal sequence of the DNA. In general, the order is as follows (fastest to slowest) :
cohesive-end ligation :
Hind III > Pst I > EcoR I > BamH I > Sal I
blunt-end ligation :
Hae III > Alu I > Hinc II >Sma I
EcoR V > Sca I > Pvu II > Nru I
The ligation rate of the restriction end of Hind III is 10-40 fold that of Sal I, and that of Hae III is 5-10 fold that of Sma I.

Quality Control

The product has passed the following quality assays: functional absence of endonuclease and exonuclease activities: ligation / recut and ligation efficiency. The enclosed buffers were assayed with the enzyme and met quality control specifications.

Protocol

Note: Before use, thaw 10× T4 DNA Ligase Buffer at room temperature and vortex vigorously to dissolve any precipitated materil.

	Cohesive Ends	Blunt Ends
10× T4 DNA Ligase Buffer	2 µl	2 µl
Insert: Vector Molar Ratio	3:1	3:1
Total DNA	0.01-0.1 µg	0.1-1.0 µg
T4 DNA Ligase	0.1 unit	1.0 unit
Autoclaved distilled water	To 20 µl	To 20 µl
Temperature	23-26°C	14°C
Time	1 H	16-24H