T4 DNA Ligase

C301

Version 21.1

Product Description

The T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the adjacent 5'-phosphate and 3'-hydroxyl on the blunt or cohesive end of dsDNA. It can also catalyze the linkage of RNA with ssDNA or RNA in double stranded nucleic acids. However, it cannot catalyze linkages between single stranded nucleotides. The T4 DNA Ligase can be used in labelling the 3'-end of RNA, cyclizing RNA and DNA oligonucleotides, cloning of cDNA, and other manipulation of nucleic acids.

Components

Components	C301-01 40,000 U
10 × Ligase Buffer*	1 ml
T4 DNA Ligase (400 U/μl)	100 μl

*It is normal if a small amount of precipitation occurs, please invert and mix before use.

Storage

Store at -30 ~ -15℃ and transport ≤0℃.

Applications

1. Ligation between DNA fragments and vector DNA.
2. Ligation between DNA fragments and Linker or Adaptor DNA.

Source

Purified from an *E.coli* with high expression of T4 DNA ligase gene.

Unit Definition

In a ligation reaction system of 20 μl, one unit (U) is defined as the amount of enzyme required to catalyze the ligation of more than 50% of 6 μg λDNA-HindIII DNA fragments in 30 min at 16℃.

Notes

For research use only. Not for use in diagnostic procedures.

Examples

1. Prepare the following reaction solution in a microcentrifuge tube:

10 × Ligase Buffer	1 μl
Insert DNA[a]	0.3 pmol
Vector DNA[b]	0.03 pmol
T4 DNA Ligase (400 U/μl)	1 μl
Sterile distilled ddH_2O	To 10 μl

a. The molar ratio of Insert/Vector should be between 3:1 and 10:1.

b. The blunt-end vector should firstly be dephosphorylated to avoid self-cycling.

2. Incubate overnight at 16℃.

3. Transformation.

① Thaw the competent cells on ice.

② Add the ligation product to 100 μl of competent cells. Gently flick the tube (Please do not oscillate) and incubate on ice for 30 min.

▲The volume of the ligation product should be less than 1/10 of the volume of competent cells.

③ Incubate the mixture at 42℃ in a water bath for exactly 45 sec. Then immediatly chill on ice for 2 - 3 min without disturbing the mixture.

④ Add 900 μl of LB or SOC medium (without antibiotics) to the centrifuge tube. Then, shake at 37℃ for 1 h at 200 - 250 rpm.

⑤ Warm selection plates to 37℃.

⑥ Centrifuge at 5,000 rpm (2,400 × g) for 5 min and discard 900 μl of supernatant. Resuspend the cells with the remaining medium and gently spread it onto a selection plate.

⑦ Incubate the plate upside down in a 37℃ incubator for 12 - 16 h.