T4 DNA Ligase

C301

Version 23.1

Product Description

T4 DNA Ligase catalyzes the formation of phosphodiester bonds between juxtaposed 5' phosphate and 3' hydroxyl termini in blunt-end or sticky-end dsDNA. This product also can catalyze the ligation of RNA and ssDNA or RNA strands in double strands, but it cannot catalyze the ligation of whole single-stranded nucleotides. It is suitable for nucleic acid operations such as labeling RNA 3'-ends, circularizing RNA and DNA oligonucleotides or cloning cDNA.

Components

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

* It is normal for Ligase Buffer to appear a small amount of precipitation when it melts, please invert and mix well before use.

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for the ligation of DNA fragments with Linker DNA, Adaptor DNA and DNA vectors.

Source

It is purified and prepared from *E. coli* with highly expressed T4 DNA ligase gene.

Unit definition

One activity unit (U) is defined as the amount of enzyme required to ligate more than 50% of the DNA fragments (In a 20 μl ligation reaction system, 6 μg of λDNA-*Hin*d III decomposition product reacts at 16°C for 30 min).

Notes

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

Examples

Ligation of DNA fragments and DNA vectors

1. Mix the following components in a microcentrifuge tube:

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

a. The molar ratio of insert to vector should be in the range of 3:1 - 10:1.

b. Please dephosphorylate the blunt-end vector to prevent self-ligation, before ligation with the insert.

2. Incubate overnight at 16°C.

3. Transformation

① Thaw chemically competent cells for cloning on ice.

② Pipette 10 μl of ligation product to 100 μl competent cells, flick the tube wall to mix thoroughly (DO NOT VORTEX!), and then place the tube on ice for 30 min.

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

③ Heat shock at 42°C water bath for 45 sec and then immediately place on ice for 2 - 3 min.

④ Add 900 μl of SOC or LB liquid medium (without antibiotics). Then, shake at 37°C for 1 h at 200 - 250 rpm.

⑤ Preheat the LB agar plate with the appropriate antibiotic at 37°C.

⑥ Centrifuge at 5,000 rpm (2,400 × g) for 5 min, discard 900 μl of supernatant. Use the remaining medium to suspend the bacteria , and then use a sterile spreading rod to gently spread on the plate with the appropriate antibiotic.

⑦ Incubate at 37°C for 12 - 16 h.