Product Manual

Product Name	T4 DNA Ligase
Source	Recombinant expression in Escherichia coli
Catalog Number	CSB-DEM049
Physical Form	Liquid
Storage Conditions	-20 ± 5°C
Molecular Weight	55.3 kDa
Quality Control	No residual exonucleases or endonucleases.
Shelf Life	24 months

Product Description

T4 DNA Ligase is an enzyme that catalyzes the formation of phosphodiester bonds between the 5'-P end of one DNA or RNA molecule and the 3'-OH end of another DNA or RNA molecule, either blunt-ended or sticky-ended. This catalytic reaction requires ATP as a cofactor. Additionally, T4 DNA Ligase can repair single-stranded nicks on double-stranded DNA, double-stranded RNA, or DNA/RNA hybrids. This product is suitable for connecting restriction enzyme-digested fragments, linkers, or adapters, as well as for nick repair and Ligase-mediated RNA detection.

Product components

Component No.	Component Name	Specifications		
1	T4 DNA ligase (400U/μL)	40KU	80KU	400KU
2	10× T4 DNA Ligase Buffer	300μL	600μL	1.5mL*2

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Operating instructions

1. **Prepare the following reaction system in a sterile microcentrifuge tube**

Composition	Usage
ddH2O	Up to 20 μL
10× T4 DNA Ligase Buffer	2 μL
T4 DNA ligase (400U/μL)	1 μL
Vector DNA	Approximately 50-100 ng
Insert fragment	Approximately 3 times the molar amount of the vector

Note:

When connecting the blunt-ended vector with DNA fragments, the vector should be dephosphorylated first to prevent self-ligation. To improve ligation efficiency, 2 μL of 50% PEG 4000 can be added to every 20 μL reaction system.

2. **Perform the reaction overnight at 16°C.**
3. **Transformation Experiment:**

(1) Add the ligation product to 100 μL of competent cells (the amount of ligation product should not exceed 1/10 of the competent cells) Gently mix by flicking and incubate on ice for 30 minutes.

(2) Heat shock at 42°C for 90 seconds, then immediately place on ice for 2-3 minutes.

(3) Add 900 μL of LB or SOC medium to the centrifuge tube and shake at 37°C and 220 rpm for 1 hour.

(4) Centrifuge at 2500g for 5 minutes, remove 900 μL of supernatant, resuspend the pellet in the remaining medium, and evenly spread the remaining cells on an appropriate antibiotic-resistant agar plate using a sterile spreader Invert the plate and incubate at 37°C overnight.