T4 DNA Ligase

Cat: T1410
Storage: -20°C to avoid repeated freezing and thawing, 10×T4 DNA Ligase Buffer is recommended for use in separate packages.
Concentration: 5U/μL
Source: Recombinant E. Coli

Kit Components:

T4 DNA Ligase	60U
10×T4 DNA Ligase Buffer	24μL

Product Description:

In the case of ATP as a coenzyme, this enzyme can catalyze the link reaction between the 5'-phosphategroup andthe 3'-hydroxyl groupof adjacent DNAchains. Both flatandsticky ends canbe used. This enzyme solution can catalyze the connection of double-stranded RNAto double-stranded DNA, but cannot catalyze the connection of single-stranded nucleic acid.

This product is mainly suitable for labeling the 3'-terminal of RNA, cyclizing RNAand DNA oligonucleotides and cloning cDNAand other nucleic acid operations.

Definition of Active Unit

In the 20μL ligating reaction system, when 6μg λDNA-Hind III decomposition reaction at 16℃ for 30min, the amount of enzyme required to catalyze more than 50% of DNAfragments for ligating was defined as 1 activity unit (U).

Examples of Use:

1. The 10×T4 DNA Ligase Buffer is melted on the ice and centrifuged briefly.
2. Using the 20μL ligase system as an example, the following ingredients are added to the sterile microcentrifuge tube:

Composition	Volume
Carrier DNA	50-100ng
Insert Fragments	The molecular molar ratio of the fragment to the carrier should be 3:1-5:1
10×T4 DNA Ligase Buffer	2μL
T4 DNA Ligase	1μL
ddH_2O	Make up to 20μL

[Note] When the flat terminal vector is connected to the DNA fragment, the vector should be dephosphorylated first to prevent self-linking phenomenon. To improve the linking efficiency, 2μL 50% PEG 4000 can be added to the reaction system every 20μL.

3. Overnight connection at 16°C.
4. Transformation experiment

(1) The junction product was added to 100μL receptive cells (the junction product should not exceed 1/10 of the receptive cells), flicked and mixed, and incubated on ice for 30 min.

(2) Set the centrifuge tube at 42°C, heat shock 90s (do not shake), and immediately place it in an

ice bath for 2-3min.

(3) 900μL LB or SOC medium was added to the centrifuge tube at 37℃, 150rpm, and oscillated for 45min.

(4) The bacteria were revived and the resistance gene was expressed during this process. Centrifuge at 2500g for 5min, 900μL supernatant was removed, the bacteria were re-suspended with the remaining medium, and the remaining bacteria were evenly coated on a correctly resistant plate with a sterile coating rod. After the bacteria solution was absorbed by the plate, the bacteria were cultured upside down at 37℃ overnight.

[Note] If super receptive cells (conversion efficiency > 10^8cfu/μg) are used, 100-200μL of bacterial solution incubated at 37℃ can be directly absorbed into the coating plate, and the remaining bacterial solution can be stored at 4℃ and can be re-coated within 1 week.

Note:

1. 10×T4 DNA Ligase Buffer contains ATP, in order to avoid degradation of ATP, it is recommended to thaw and freeze at -20℃. If a small amount of precipitation occurs in the 10×T4 DNA Ligase Buffer during melting, please mix it upside down and use it.
2. When the flat terminal vector is connected to the DNA fragment, the vector should be dephosphorylated first to prevent the vector from cyclizing itself.

Note: If PEG is added to the reaction system, it can promote the binding of flat ends, but PEG may cause the formation of concatenates in the cDNA fragment clones and inhibit the packaging reaction.

3. For your safety and health, wear a lab coat and wear disposable gloves during operation.
4. This product is for scientific research purposes only!

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