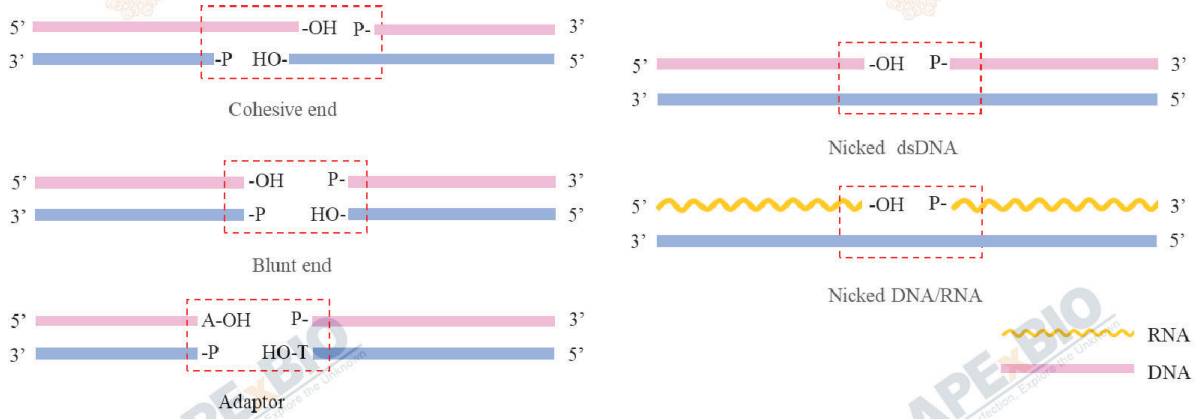
T4 DNA Ligase (RNase-free)

Product description

T4 DNA Ligase is a single-stranded polypeptide with a molecular weight of 55.3 kDa, which can catalyze the formation of phosphodiester bonds at adjacent 5' phosphate and 3' hydroxyl ends on double-stranded DNA or RNA. The ligase has no catalytic activity for single-stranded nucleic acids, and its catalytic reaction requires ATP as a cofactor.

T4 DNA ligase is versatile, can not only bind DNA fragments to cohesive or blunt ends, but also repair single-stranded nicks on double-stranded DNA, double-stranded RNA, or DNA/RNA hybrids. It is commonly used for connection of DNA fragments with vectors, linkers or adaptors; nicks repair; and linear DNA self-cyclization.



Linkage substrate for T4 DNA ligase

Composition and storage conditions

Components \ Size	40 KU	100 KU	200 KU
T4 DNA Ligase (400 U/μL)	0.1 mL	0.25 mL	0.5 mL
10X T4 DNA Ligase Reaction Buffer	1 mL	2.5 mL	5 mL
Store the components at -20 °C.			

Experimental operation

Take ligation of DNA fragment and vector, transforming E. coli as an example.

1. Configure the reaction system on ice according to the following table:

Total Reaction Volume	20 μL
10X T4 DNA Ligase Reaction Buffer	2 μL
Vector DNA (5 kb)	50 ng
Insert DNA (1 kb)	30 ng
T4 DNA Ligase	1 μL
Nuclease-free Water	to 20 μL

2. Mix the reaction system gently (pipette to mix by blowing or vortexing at lowest speed).
3. Incubate at 20-25 °C for 1-2 h, or incubate at 16 °C overnight. (In order to quickly obtain the intended clones, 5 μL of reaction solution can be first transformed into E. coli after 1-2 h incubation at 20-25 °C, and the remaining 15 μL of reaction solution can be incubated overnight at 16 °C. If the clone is successfully obtained the next day, it can proceed to the next step. Otherwise, the remaining reaction products incubated overnight can be transformed into E. coli again.)
4. Transform

1) Add the reaction product to a centrifuge tube containing 100 μL of competent E. coli, mix gently, and incubate on ice for 30 min. Note that the volume of reaction products should not exceed 1/6 of the volume of competent cells.
2) Place the centrifuge tube in a 42 °C water bath without shaking. After accurate heat shock for 90 s, immediately stand it in an ice water bath for 2-3 min.
3) Add 500 μL of LB or SOC medium to the centrifuge tube, and incubate at 37 °C with shaking at 150 rpm for 45-60 min to revive the bacteria and express resistance genes.
4) Centrifuge at 5000 rpm (2500×g) for 5 min and remove 400 μL of supernatant. Resuspend the E. coli with remaining medium, and then coat the medium with a sterile coating stick on culture plate containing the corresponding resistance. Let stand at room temperature for 10 min.
5) After the medium is absorbed by the culture plate, the culture plate is inverted at 37 °C overnight.

Notes

1. Usually 1 μL ligase is sufficient for each reaction. If you want to further improve ligation efficiency, the amount of ligase can be increased appropriately.
2. It is recommended that the molar ratio of the inserted fragment and the vector be between 3:1-10:1. In addition, when making TA ligation, the molar concentration ratio of dA-Tailing's DNA product with ‘T’sticky end's adaptor should be between 1:10-1:20.
3. When attaching a blunt-end vector to the DNA fragment, the vector should first be dephosphorylated at 5’-terminal to prevent its self-cyclization.
4. T4 DNA Ligase is unstable, so it must be operated on ice and stored at -20 °C immediately after use.

Product description

1. Enzyme activation unit (U) definition: 50 μL reaction system containing T4 DNA ligase reaction buffer, reaction at 16 °C for 30 minutes, 1 U of T4 DNA ligase link the HindIII-digested lambda DNA fragments (5’ end concentration of DNA 0.12 μM (300 ng/mL)) to 50%.

2. Stored solution composition: 10 mM Tris-HCl (pH 7.4), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol.
3. 10x T4 DNA Ligase Reaction buffer: 500 mM Tris-HCl (pH 7.5 @ 25 °C), 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP.
4. Quality assurance:
   - Purity: SDS-PAGE detection purity > 95%.
   - Non-specific nuclease: 2000 U of this enzyme was incubated with Lambda DNA for 16 h at 37 °C without changing the electrophoresis band.
   - Non-endonuclease: 2000 U of this enzyme was incubated with 1 μg of supercoiled PhiX174 DNA for 16 h at 37 °C, the DNA de-supercoiled < 10%.
   - Non-exonuclease: 2000 U of this enzyme was incubated with HindIII-DNA for 16 h at 37 °C without changing the electrophoresis band.
   - Non-RNase: 400 U of this enzyme and 200 ng of 300 base RNA substrate were reacted for 16 h at 37 °C without changing the electrophoresis band of RNA.
5. Inactivation conditions: Incubation at 65 °C for 10 min can result in inactivation of T4 DNA Ligase.
6. This product is for scientific use only.

Troubleshooting

1. Substrate linkage failed

Probable Causes	Corresponding Suggestion
Poor quality of ATP and Mg^{2+} within the reaction system	Use the kit as soon as possible after purchase. ATP in this kit may have degraded over a year. In addition, if supplement ATP on your own, be sure to use ribose ATP, because deoxyribose ATP won't work.
High concentration of DNA produces only linear DNA	The total DNA concentration should be maintained between 1-10 μg/mL.
The insert is too large	Reduce the insert concentration and then incubate overnight at 16 °C using a concentrated ligasse.
Ligases are inactive	The ligases activity can be tested with λDNA-HindIII digested substrates or other suitable substrates.

2. Cell transform failed

Probable Causes	Corresponding Suggestion
Recombinant is too large (> 10,000 bp)	Excessive size of recombinant may result in the inability of chemically prepared competent cells, and electroporation can be attempted for transforming.
Too much ligation product is added to the cell	The amount of reaction product added in 50 μL of competent cells should be controlled at 1-5 μL.
The insert contains methylated cytosine	The methylated cytosine sequence is degraded by many E. coli strains. Try using strains lacking mcrA, mcrBC and MRR for transforming.
E. coli does not tolerate	Try using the pMAL protein fusion expression system for protein recombination, or try

recombinant proteins well	another expression system that does not involve E. coli.