T4 DNA Ligase (Fast)

Instruction for Use

Cat. No./Spec.: K012/1000 U

Concentration: 5 U/μL

Note: 1 U=1 Weiss unit

Product Description

T4 DNA Ligase (Fast) is produced by *E. coli* carrying T4 bacteriophage gene 30. This enzyme catalyzes the formation of phosphodiester bonds between the 5'-phosphate group and the 3'-hydroxyl group of double-helix DNA or RNA. This enzyme can repair single-strand breaks in double-stranded DNA, RNA, or DNA/RNA complexes, and can connect DNA fragments with sticky or blunt ends, but it is inactive for single-stranded nucleic acids. It is mainly used for cloning of restriction enzyme digestion products, gene site-directed mutagenesis, PCR product cloning, linear DNA self-looping, and repair of double-stranded DNA breaks. T4 DNA Ligase (Fast) requires ATP as a cofactor and completes the sticky end ligation reaction at room temperature in only 10 minutes.

Components

Component	K012 (1,000 U)
T4 DNA Ligase (Fast) (5 U/μL)	200 μL
10X T4 DNA Ligase Buffer	1 mL × 2
50% PEG	1 mL

Storage Condition

Store at -20°C.

Unit Definition

At 37°C, 1 Weiss unit of enzyme catalyzes the conversion of 1 nmol of [^{32}PPi] into an activated carbon adsorption state within 20 minutes. One Weiss unit is equivalent to approximately 200 cohesive end ligation units (CEU), which is equivalent to the number of HindIII digested DNA fragments that can be ligated within 30 minutes at 16°C. λ DNA fragments.

Enzyme Activity Detection Conditions

The enzyme activity was tested in the following reaction mixture: 66 mM Tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 66 μM ATP, 10 mM DTT, and 3.3 μM [^{32}PPi].

Quality Control

Nuclease residue detection: at 37°C, 200 U of T4 DNA Ligase (Fast) was incubated with 1 μg of pUC19 DNA for 4 hours, and no conversion of covalently closed circular DNA to DNA with nick was detected.

Nuclease residue detection: the enzyme solution was incubated with double-stranded DNA substrate at 37°C for 16 hours, and no changes were detected in the double-stranded DNA substrate by DNA electrophoresis.

Blue-white colony test: at room temperature, 30 U of T4 DNA Ligase (Fast) was used to ligate the HindIII, PstI, or SmaI digested pUC57 DNA for 1 hour. Then, the ligation products were transformed into *E. coli* XL1-Blue competent cells, and less than 1% of white colonies were detected.

Protocol

1. Ligation of DNA Insertion Fragment to Vector DNA (Sticky End Ligation)

① Prepare the following reaction system on ice:

Component	Amount
Linear vector DNA	20~100 ng
Insert DNA	3:1~10:1 (molar ratio of Fragment: Vector)
10X T4 DNA Ligase Buffer	2 μL
T4 DNA Ligase (Fast) (5 U/μL)	1 U (0.2 μL)
ddH_2O	To 20 μL

② Mix well and centrifuge briefly, incubate at 22℃ for 10 minutes;

③ Take 1-5 μl the ligation product for transformation of 50 μl chemically competent cells, or taking 1~2 μl of the ligation product for transformation of 50 μl electro-competent cells.

Note: If the reaction products are used for electrical transformation, a centrifuge column or chloroform extraction should be used to clean DNA instead of thermal inactivation.

2. Ligation of DNA Insertion Fragment to Vector DNA (Blunt End Ligation)

① Prepare the following reaction system on ice:

Component	Amount
Linear vector DNA	20~100 ng
Insert DNA	3:1~10:1 (molar ratio of Fragment: Vector)
10X T4 DNA Ligase Buffer	2 μL
50% PEG	2 μL
T4 DNA Ligase (Fast) (5 U/μL)	5 U (1 μL)
ddH_2O	To 20 μL

② Mix well and centrifuge briefly, incubate at 22℃ for 1 hour;

③ Take 1-5 μl the ligation product for transformation of 50 μl chemically competent cells, or taking 1~2 μl of the ligation product for transformation of 50 μl electro-competent cells.

Note: If the reaction products are used for electrical transformation, a centrifuge column or chloroform extraction should be used to clean DNA instead of thermal inactivation.

3. Linear DNA Self-cycling

① Prepare the following reaction system on ice:

Component	Amount
Linear DNA	10~50 ng
10X T4 DNA Ligase Buffer	5 μL
T4 DNA Ligase (Fast) (5 U/μL)	5 U (1 μL)
ddH_2O	To 50 μL

② Mix well and centrifuge briefly, incubate at 22℃ for 10 minutes;

③ Take 1-5 μl the ligation product for transformation of 50 μl chemically competent cells, or taking 1~2 μl of the ligation product for transformation of 50 μl electro-competent cells.

Note: If the reaction products are used for electrical transformation, a centrifuge column or chloroform extraction should be used to clean DNA instead of thermal inactivation.

4. Adaptor Ligation

Double-stranded oligonucleotide linkers are often used to create sticky ends on the inserted fragment. The adaptor usually contains restriction enzyme recognition sites, which are cleaved after ligation to produce sticky ends that match the cloning vector. Sometimes the adaptors already contain sticky ends that match the cloning vector, in which case there is no need to further process the inserted fragment after the adaptor ligation is complete.

① Prepare the following reaction system on ice:

Component	Amount
Linear DNA	100~500 ng
Phosphorylated adaptor	1~2 μg
10X T4 DNA Ligase Buffer	2 μL
50% PEG	2 μL
T4 DNA Ligase (Fast) (5 U/μL)	2 U (0.4 μL)
ddH_2O	To 20 μL

② Mix well and centrifuge briefly, incubate at 22℃ for 1 hour;

③ Performe heat inactivation at 65℃ for 10 minutes or at 70℃ for 5 minutes.

Notes

1. T4 DNA Ligase (Fast) is strongly inhibited in the presence of NaCl or KCl concentrations higher than 200 mM.
2. The volume of the ligation reaction solution should not exceed 10% of the competent cell volume, and it is not recommended to add excess volume to the system.
3. DNA bound to T4 DNA Ligase (Fast) may migrate abnormally or spread in agarose gel electrophoresis, in order to avoid this phenomenon, the enzyme can be heat-inactivated before loading if necessary, and add a suitable amount of SDS if necessary.
4. Polyethylene glycol (PEG) can greatly improve the efficiency of flat-end ligation, and the recommended addition amount of PEG 8000 is 5% (w/v) of the ligation system.
5. Electrical transformation efficiency may be improved by heat-inactivating T4 DNA Ligase (Fast) or using centrifuge columns or chloroform extraction to purify DNA.
6. The number of transformants can be increased by extending the reaction time to 1 hour.