Fast T4 DNA Ligase

Cat #: C-BSM205

Size: 1000 U

Storage: -20°C

Components

Components	Size
Fast T4 DNA Ligase (5 U/μl)	200 μl
10×T4 DNA Ligase Buffer	1 ml
50% PEG	1 ml

Note: 1 U=1 Weiss unit

Product Introduction

Fast T4 DNA Ligase is produced by Escherichia coli carrying the T4 phage gene 30. This enzyme catalyzes the formation of phosphodiester bonds between the 5'-phosphate group and the 3'-hydroxyl group of double-stranded DNA or RNA. It can repair single-strand nicks in double-stranded DNA, RNA, or DNA/RNA hybrids, and can ligate DNA fragments with cohesive or blunt ends. However, it is inactive on single-stranded nucleic acids. It is mainly used for cloning of restriction enzyme-digested DNA fragments, site-directed mutagenesis, cloning of PCR products, and repair of double-strand DNA nicks. Fast T4 DNA Ligase requires ATP as a cofactor and can complete sticky-end ligation reactions at room temperature in just 10 minutes.

Enzyme Activity Definition

Under 37°C, 1 Weiss unit of the enzyme catalyzes the conversion of 1 nmol of [32PPi] to adsorbed active carbon within 20 minutes. 1 Weiss unit is equivalent to approximately 200 cohesive-end ligation units (CEUs), which is similar to the ligation of 50% HindIII-digested λDNA fragments within 30 minutes at 16°C.

Quality Control

Residual endonuclease activity detection:

At 37°C, incubate 200 U of Fast T4 DNA Ligase with 1 μg of pUC19 DNA for 4 hours. No conversion from covalently closed circular DNA to nicked DNA is detected.

Residual exonuclease activity detection:

Incubate the enzyme solution with double-stranded DNA substrate at 37°C for 16 hours. No change in the double-stranded DNA substrate is detected by DNA gel electrophoresis.

Blue/white screening:

At room temperature, incubate pUC57 DNA/HindIII, pUC57 DNA/PstI, or pUC57 DNA/SmaI digestion products with 30 U of Fast T4 DNA Ligase for 1 hour. Then transform the ligation products into competent E.coli XL1-Blue cells. Less than 1% of white colonies are detected.

Instructions for use:

1. Connection of DNA insert fragment to vector DNA (sticky-end ligation):

① Prepare the following reaction mixture on ice:

Components	Volume
Linearized vector DNA	20-100 ng
Insert fragment DNA	3:1-10:1 (Fragment-to-vector molar ratio)
10×T4 DNA Ligase Buffer	2 μl
Fast T4 DNA Ligase	1 U (0.2 μl)
Nuclease-Free Water	Up to 20 μl

② Mix thoroughly and briefly centrifuge, then incubate at 22°C for 10 minutes.

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

Note: If the ligation product is used for electroporation, use column purification or chloroform extraction to clean the DNA instead of the heat inactivation step.

2. Connection of DNA insert fragment to vector DNA (blunt-end ligation)

① Prepare the following reaction mixture on ice:

Components	Volume
Linearized vector DNA	20-100 ng
Insert fragment DNA	3:1-10:1 (Fragment-to-vector molar ratio)
10×T4 DNA Ligase Buffer	2 μl
50% PEG	2 μl
Fast T4 DNA Ligase	5 U (1 μl)
Nuclease-Free Water	Up to 20 μl

② Mix thoroughly and briefly centrifuge, then incubate at 22°C for 1 hour.

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

Note: If the ligation product is used for electroporation, use column purification or chloroform extraction to clean the DNA instead of the heat inactivation step.

3. Linear DNA self-circularization

① Prepare the following reaction mixture on ice:

Components	Volume
Linearized DNA	10-50 ng
10×T4 DNA Ligase Buffer	5 μl
Fast T4 DNA Ligase	5 U (1 μl)
Nuclease-Free Water	Up to 50 μl

② Thoroughly mix and briefly centrifuge, then incubate at 22°C for 10 minutes.

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

Note: If the ligation product is used for electroporation, use column purification or chloroform extraction to clean the DNA instead of the heat inactivation step.

4. Adapter ligation

Double-stranded oligonucleotide adapters are often used to generate sticky ends on the insert fragment. The adapters usually contain restriction enzyme recognition sites that, after ligation and enzyme digestion, generate compatible sticky ends with the cloning vector. Sometimes the adapters already contain sticky ends compatible with the cloning vector, eliminating the need for further processing of the insert fragment after adapter ligation.

① Prepare the following reaction mixture on ice:

Components	Volume
Linearized DNA	100-500 ng
Phosphorylated adapter	1-2 µg
10×T4 DNA Ligase Buffer	2 µl
50% PEG	2 µl
Fast T4 DNA Ligase	2 U (0.4 µl)
Nuclease-Free Water	Up to 20 µl

② Thoroughly mix and briefly centrifuge, then incubate at 22°C for 10 minutes.

③ Perform heat inactivation at 65°C for 10 minutes or 70°C for 5 minutes.

Note 1: Fast T4 DNA Ligase has 100% activity in CutOne™ restriction enzyme buffer in the presence of 1 mM ATP. Therefore, the adapter ligation reaction can be performed in CutOne™ restriction enzyme buffer to simplify the "adapter ligation-enzyme digestion" experimental workflow. The specific method is as follows: Add ATP to a final concentration of 1 mM in the adapter ligation reaction mixture, then inactivate Fast T4 DNA Ligase after the adapter ligation is complete. Finally, add an appropriate amount of FlyCut™ Fast Restriction Enzyme to the mixture and incubate at the optimal enzyme digestion temperature.

Note 2: CutOne™ restriction enzyme buffer does not contain ATP component.

Notes

- Fast T4 DNA Ligase is strongly inhibited by NaCl or KCl concentrations higher than 200 mM.
- The amount of ligation reaction mixture should not exceed 10% of the volume of competent cells, and excessive Fast T4 DNA Ligase is not recommended in the system.

- DNA bound to Fast T4 DNA Ligase may exhibit band shifting or smearing on agarose gels. To avoid this phenomenon, the enzyme can be heat-inactivated before loading, and if necessary, an appropriate amount of SDS can be added.
- Polyethylene glycol (PEG) significantly improves the efficiency of blunt-end ligation. The recommended concentration of PEG 8000 is 5% (w/v) of the ligation mixture.
- Electroporation efficiency can be improved by heat inactivation of Fast T4 DNA Ligase or by using column purification or chloroform extraction for DNA purification.
- The number of transformants can be increased by extending the reaction time to 1 hour.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.