T4 DNA Ligase(Fast)

REF: EG15205S

Storage Condition

-20℃

Components

Component	Amount
T4 DNA Ligase(Fast) (5 U/μl)	200 μl
10×T4 DNA Ligase Buffer	2×1 ml
50% PEG	1 ml

Note: 1 U=1 Weiss unit

Description

T4 DNA Ligase(Fast) is produced by *E.coli* carrying T4 bacteriophage gene 30. The enzyme catalyzes the formation of phosphodiester bonds between 5'-phosphate groups and 3'-hydroxyl groups 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 sticky or blunt ends. It is not active on single-stranded nucleic acids. The enzyme is primarily used for cloning of restriction endonuclease-digested DNA fragments, site-directed mutagenesis, cloning of PCR products, linear DNA circularization, and repair of double-stranded DNA nicks. T4 DNA Ligase (Fast) requires ATP as a cofactor and completes sticky end ligation reactions at room temperature in just 10 minutes.

Definition of Activity Unit

Under 37℃ conditions, 1 Weiss unit of the enzyme catalyzes the conversion of 1 nmol of [^{32}PPi] into Norit-adsorbable form within 20 minutes at 37 ℃ . One Weiss unit is approximately equivalent to 200 cohesive end ligation units (CEU), which corresponds to the ligation of λDNA fragments digested with 50% HindIII in 30 minutes at 16℃ .

Quality Control Assays

Endonuclease Activity

A 20 μl reaction in T4 DNA Ligase containing 200 ng of supercoiled plasmid and 5 U of T4 DNA Ligase (Fast) incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Blue/White Screening

5 U of T4 DNA Ligase (Fast) was used to ligate pUC57 DNA/HindIII, pUC57 DNA/PstI, or pUC57 DNA/SmaI digested products for 1 hour at 22℃ . The resulting ligation products were then transformed into *E.coli* XL1-Blue competent cells. On Luria-Bertani culture plate with X-Gal, IPTG and appropriate antibiotic, less than 1% of colonies showed white spots.

Protocol

1. Connecting DNA Insert Fragment to Vector DNA (Sticky end)

① Prepare the following reaction mixture on ice:

Reagent	Amount
Linearized vector DNA	20~100 ng
Insert fragment DNA	3:1~10:1 (molar ratio of fragment to vector)
10×T4 DNA Ligase Buffer	2 μl
T4 DNA Ligase(Fast)	1 U (0.2 μl)
Nuclease-Free Water	To 20 μl

② Mix gently and spin down, then incubate at 22℃ for 10 minutes;

③ Take 1~5 μl of the ligation product for transforming chemically competent cells in a 50 μl reaction, or take 1~2 μl for transforming electroporation-competent cells in a 50 μl reaction.

Note: If the ligation product is used for electrophoresis, perform a purification step instead of the heat inactivation step.

2. Connecting DNA Insert Fragment to Vector DNA (Blunt end)

① Prepare the following reaction mixture on ice:

Reagent	Amount
Linearized vector DNA	20~100 ng
Insert fragment DNA	3:1~10:1 (molar ratio of fragment to vector)
10×T4 DNA Ligase Buffer	2 μl
50% PEG	2 μl
T4 DNA Ligase(Fast)	5 U (1 μl)
Nuclease-Free Water	To 20 μl

② Mix gently and spin down, then incubate at 22℃ for 1 hour;

③ Take 1~5 μl of the ligation product for transforming chemically competent cells in a 50 μl reaction, or take 1~2 μl for transforming electroporation-competent cells in a 50 μl reaction.

Note: If the ligation product is used for electrophoresis, perform a purification step instead of the heat inactivation step.

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3. Linear DNA Circularization

① Prepare the following reaction mixture on ice:

Reagent	Amount
Linearized DNA	10~50 ng
10×T4 DNA Ligase Buffer	5 μl
T4 DNA Ligase(Fast)	5 U (1 μl)
Nuclease-Free Water	To 50 μl

② Mix gently and spin down, then incubate at 22℃ for 10 minutes;

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

Note: If the ligation product is used for electrophoresis, perform a purification step instead of the heat inactivation step.

4. Adapter Ligation

Double-stranded oligonucleotide adapters are often used to generate sticky ends on insert fragments. Adapters typically contain restriction enzyme recognition sites that, after ligation and enzyme digestion, generate sticky ends compatible with the cloning vector. In some cases, the adapter already contains 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:

Reagent	Amount
Linearized DNA	100~500 ng
Phosphorylated adapters	1~2 μg
10×T4 DNA Ligase Buffer	2 μl
50% PEG	2 μl
T4 DNA Ligase(Fast)	2 U (0.4 μl)
Nuclease-Free Water	To 20 μl

② Mix gently and spin down, then incubate at 22℃ for 10 minutes;

③ Perform heat inactivation by incubating at 65℃ for 10 minutes or 70℃ for 5 minutes.

Note 1: T4 DNA Ligase (Fast) has 100% activity in the presence of 1 mM ATP in CutOne™ buffer. Therefore, the adapter ligation reaction can be performed in CutOne™ buffer, simplifying the "adapter ligation- digestion" experimental workflow. The specific method is as follows: add ATP to a final concentration of 1 mM to the adapter ligation mixture, inactivate T4 DNA Ligase (Fast) after adapter ligation is complete. Then, add an appropriate amount of LightNing™ restriction enzymes to the mixture, and finally, incubate at the optimal digestion temperature.
Note 2: CutOne™ buffer does not contain ATP. We provides an independent package of 100 mM ATP stock solution (REF: EG21916), which is available for purchase.

Notice

1. T4 DNA Ligase (Fast) is strongly inhibited by NaCl or KCl concentrations above 200 mM;
2. The amount of ligation reaction mixture should not exceed 10% of the volume of competent cells. It is not recommended to add an excessive amount of T4 DNA Ligase (Fast);
3. DNA bound to T4 DNA Ligase (Fast) may exhibit band shifting or smearing on agarose gel. To avoid this phenomenon, the enzyme can be heat inactivated before loading, and a suitable amount of SDS can be added if necessary;
4. Polyethylene glycol (PEG) significantly enhances the efficiency of blunt-end ligation. The recommended concentration of PEG 8000 is 5% (w/v) of the ligation system;
5. Electrophoresis efficiency can be improved by heat inactivating T4 DNA Ligase (Fast), or purifying the ligation products;
6. The number of transformants can be increased by extending the ligation time to 1 hour.

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