MCE USA
Tel: 609-228-6898
Email: tech@MedChemExpress.com

T4 DNA Ligase (Fast)

1 Components

Components	HY-KD1011-100 T
T4 DNA Ligase (Fast) (5U/μL)	200 μL
10 × T4 DNA Ligase Buffer	1 mL
50% PEG	1 mL

Note：1 U=1 Weiss unit

2 Introduction

MCE T4 DNA Ligase (Fast) is produced by Escherichia coli carrying a T4 phage, catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. The enzyme joins DNA fragments with either cohesive or blunt termini, repairs single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids, but it is inactive against single-stranded nucleic acids. It is suitable for labeling RNA 3'-ends, cyclizing RNA and DNA oligonucleotides and cloning cDNA. The T4 DNA Ligase requires ATP as a cofactor, completes viscous end connections in just 10 min at room temperature. Simple handling for fast and efficient connections.

3 Definition of Activity Unit

One Weiss unit of the enzyme catalyzes the conversion of 1 nmol of [^{32}PPi] into Norit-adsorbable form in 20 min at 37°C. One Weiss unit is equivalent to approximately 200 cohesive end ligation units (CEU), which corresponds to the amount of enzyme required to give 50% ligation of HindIII fragments of lambda DNA in 30 min at 16°C.

Enzyme activity is assayed in the following mixture: 66 mM Tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 66 μM ATP, 10 mM DTT, 3.3 μM [^{32}PPi].

4 Quality Control

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 200 Weiss units of T4 DNA Ligase with 1 μg of pUC19 DNA for 4 h at 37°C.

Ribonuclease Assay

No degradation of double-stranded labeled oligonucleotide was observed after incubation with T4 DNA Ligase (Fast) for 16 h at 37°C.

Blue/White (B/W) Cloning Assay

pUC 57 DNA-Hind III, pUC57 DNA-Pst I or pUC57 DNA-San I digestion products were incubated with 30 U T4 DNA Ligase (Fast) for 1 h at room temperature. The ligated products were transformed with E.coli XL 1-Blue receptor cells, less than 1% white spots were detected.

5 General Protocol

DNA Insert Ligation (sticky-end and blunt-end) into Vector DNA

1.Prepare the following reaction mixture (operation on ice).

Sticky-end Ligation

Reagent	Amount
Vector DNA	20-100 ng
Insert DNA	3:1 - 10:1 (mole ratio) VS Vector
10 × T4 DNA Ligase Buffer	2 μL
T4 DNA Ligase (Fast) (5U/μL)	1 U (0.2 μL)
ddH_2O	To 20 μL

Blunt-end Ligation

Reagent	Amount
Vector DNA	20-100 ng
Insert DNA	3:1-10:1 (mole ratio) VS Vector
50% PEG	2 μL
10 × T4 DNA Ligase Buffer	2 μL
T4 DNA Ligase (Fast) (5U/μL)	5 U (1 μL)
ddH_2O	To 20 μL

Note: If the vector is single digested, dephosphorylate the digested clone to prevent the formation of additional self-associated clones of the vector.

2.Mix thoroughly, and spin briefly. Incubate 10 min at 22°C for sticky-end ligation and 1 h at 22°C for blunt-end Ligation.

3.Use 1-5 μL of the mixture for transformation of 50 μL chemically competent cells and 1-2 μL per 50 μL of electrocompetent cells.

Note: If the ligation reaction mixture will be used for electroporation, replace the heat inactivation step with spin column purification or chloroform extraction.

Self-circularization of Linear DNA

1.Prepare the following reaction mixture (operation on ice).

Self-circularization of Linear DNA

Reagent	Amount
Linear DNA	10-50 ng
10 × T4 DNA Ligase Buffer	5 μL
T4 DNA Ligase (Fast) (5U/μL)	5 U (1 μL)
ddH_2O	To 50 μL

2.Mix thoroughly, spin briefly and incubate for 10 min at 22°C.

3.Use 1-5 μL of the mixture for transformation 50 μL of chemically competent cells or 1-2 μL of the mixture for transformin 50 μL of electrocompetent cells.

Note: If the ligation reaction mixture is intended for electroporation, replace the heat inactivation step with spin column purification or chloroform extraction.

Linker Ligation

Double stranded oligonucleotide linkers are often used to generate compatible overhangs that are not found in the insert. There linkers typically contain restriction enzyme recognition sequences and are digested after ligation to generate overhangs compatible with cloning vectors. Alternatively, linkers may have overhangs which are ready for ligation with a cloning vector and do not require further manipulation following ligation.

1.Prepare the following reaction mixture (operation on ice).

Linker Ligation

Reagent	Amount
Linear DNA	100-500 ng
Phosphorylated linkers	1-2 μg
50% PEG	2 μL
10 × T4 DNA Ligase Buffer	2 μL
T4 DNA Ligase (Fast) (5U/μL)	2 U (0.4 μL)
ddH_2O	To 20 μL

2.Mix thoroughly, spin briefly and incubate for 10 min at 22°C.

3.Heat inactivate at 65°C for 10 min or at 70°C for 5 min.

Note: Linker ligation reactions can be performed in the restriction enzyme buffer optimal for the subsequent digestion. In this case, the ligation reaction should be supplemented with ATP to a final concentration of 1 mM. After inactivation of the T4 DNA Ligase, add the restriction enzyme directly to the reaction mixture and incubate according to the digestion protocol.

6 Storage

-20°C, 1 year

7 Precautions

1.It is normal for a small amount of precipitate to appear when the Buffer is melted, please reverse the mixing process before use.

2.Enzymes should be stored in an ice box or on an ice bath and stored at -20°C immediately after use.

3.T4 DNA Ligase is strongly inhibited by NaCl or KCl at concentrations higher than 200 mM.

4.For efficient transformation, the volume of the ligation reaction mixture should not exceed 10% of the competent cell volume. It is not recommended to add excess T4 DNA Ligase (Fast) to the system.

5.Binding of T4 DNA Ligase to DNA may result in a band shift in agarose gels. To avoid this, incubate samples with 6 × Loading Dye & SDS Solution at 65°C for 10 min and chill on ice prior to loading.

6.Polyethylene glycol (PEG) greatly increases the ligation efficiency of blunt-end DNA ligation. The recommended concentration of PEG 8000 in the ligation reaction mixture is 5% (w/v).

7.This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

8.For your safety and health, please wear a lab coat and disposable gloves to operate.