T4 DNA Ligase, LC

Instruction for Use

Cat. No./Spec.: E1014-E/1,000 Weiss U

Concentration: 1 Weiss U/μL

Product Description

T4 DNA Ligase catalyzes the formation of phosphodiester bonds between juxtaposed 5'-phosphate and 3'-hydroxyl termini in double-stranded DNA or RNA. This enzyme is capable of repairing single-strand nicks in double-stranded DNA, RNA, or DNA/RNA hybrids, and can join DNA fragments with sticky or blunt ends, but it is inactive on single-stranded nucleic acids.

T4 DNA Ligase requires ATP as a cofactor.

Components

Component	*E1014-E*
T4 DNA Ligase, LC (1 Weiss U/μL)	*500 μL × 2*
10X T4 DNA Ligase Buffer	*1.5 mL*
50% PEG	*1.5 mL*

Storage Condition & Shelf Life

Store at -20°C. The product is valid for 2 years.

Source

Recombinant E. coli strain containing the gene 30 cloned from bacteriophage T4.

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.

Enzyme activity is determined in the following mixture: 66 mM Tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 0.066 mM ATP, 10 mM DTT, 3.3 μM [^{32}P]orthophosphate (PPi).

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.

Scope of Application

- DNA fragments generated by cloning restriction enzymes

- Cloned PCR products

- Ligation of double-stranded oligonucleotides or adapters to DNA

- Site-directed mutagenesis

- Amplified fragment length polymorphism (AFLP)

- Ligase-mediated RNA detection

- Nick repair in double-stranded DNA, RNA, or DNA/RNA hybrids

- Self-circularization of linear DNA

Inhibition and Inactivation

- The activity of T4 DNA Ligase is strongly inhibited when the concentration of NaCl or KCl is higher than 200 mM.

- T4 DNA Ligase will become inactivated by heating at 65°C for 10 minutes or at 70°C for 5 minutes.

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	*1:1~5:1 (molar ratio of Fragment: Vector)*
10X T4 DNA Ligase Buffer	*2 μL*
T4 DNA Ligase	*1 Weiss U*
ddH_2O	*To 20 μL*

② *Mix well and centrifuge briefly, incubate at 22˚C 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:

- The following methods can be employed to improve the efficiency of electroporation:

1. Inactivate T4 DNA Ligase by heating at 65°C for 10 minutes or at 70°C for 5 minutes.

2. Purify DNA using a PCR purification kit or chloroform extraction.

- The total number of transformants can be increased by extending the reaction time to one hour.

- If more than 2 Weiss units of T4 DNA Ligase are used in a 20-microliter reaction mixture, DNA must be purified by centrifugal column or chloroform extraction before electroporation.

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	*1:1~5:1 (molar ratio of Fragment: Vector)*
10X T4 DNA Ligase Buffer	*2 μL*
50% PEG	*2 μL*
T4 DNA Ligase	*5 Weiss U*
ddH_2O	*To 20 μL*

② *Mix well and centrifuge briefly, incubate at 22˚C 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	*5 Weiss U*
ddH_2O	*To 50 μL*

② *Mix well and centrifuge briefly, incubate at 22˚C 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:

- The following methods can be employed to improve the efficiency of electroporation:

1. Inactivate T4 DNA Ligase by heating at 65°C for 10 minutes or at 70°C for 5 minutes.

2. Purify DNA using a PCR purification kit or chloroform extraction.

- The total number of transformants can be increased by extending the reaction time to one hour.

Important Notes:

- Polyethylene glycol (PEG) can significantly enhance the efficiency of blunt-end DNA ligation. The recommended concentration of PEG 4000 in the ligation reaction mixture is 5% (w/v).

- Do not exceed the recommended amount of T4 DNA Ligase in the reaction mixture.

- The binding of T4 DNA Ligase to DNA may cause band migration in agarose gels. To avoid this, incubate the samples with 6× Gel Loading Dye, SDS+ (#M9081) at 65°C for 10 minutes before loading, and then cool on ice.

- To achieve efficient transformation, the volume of the ligation reaction mixture should not exceed 10% of the volume of competent cells.

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 Linker	*1~2 μg*
10X T4 DNA Ligase Buffer	*2 μL*
50% PEG	*2 μL*
T4 DNA Ligase	*2 Weiss U*
ddH_2O	*To 20 μL*

② *Mix well and centrifuge briefly, incubate at 22˚C for 1 hour;*

③ *Performe heat inactivation at 65˚C for 10 minutes or at 70˚C for 5 minutes.*

This product is for research use only.