T4 DNA Ligase, 5 U/μL

FB02503100 200 μL

Stored at -25°C to -15°C

T4 DNA Ligase catalyzes phosphodiester bond formation between 5'-phosphate and 3'-hydroxyl termini in duplex DNA/RNA, repairing single-strand nicks and joining DNA fragments with either cohesive or blunt termini. The reaction requires ATP as a cofactor.

Applications:

- Cloning of restriction enzyme generated DNA fragments.
- Cloning of PCR products.
- Joining double-stranded oligonucleotide linkers or adaptors to DNA.
- Site-directed mutagenesis.
- Amplified fragment length polymorphism (AFLP).
- Ligase-mediated RNA detection.
- Nick repair in duplex DNA, RNA or DNA/RNA hybrids.
- Self-circularization of linear DNA.

Kit Contents

Components	Volume
T4 DNA Ligase, 5 U/μL	1000 U
10X T4 DNA Ligase Buffer	1.5 mL
50% PEG Solution	1.5 mL

Definition of Activity Unit

One unit is equivalent to approximately 200 cohesive end ligation units (CEU). One CEU is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of lambda DNA in 30 min at 16°C.

DNA INSERT LIGATION INTO VECTOR DNA

Sticky-end ligation

1. Prepare the following reaction mixture:

Component	20 μL Reaction
Linear vector DNA	20-100 ng
Insert DNA:	1:1 to 5:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2 μL
T4 DNA Ligase	1 U
Water, nuclease-free	to 20 μL

2. Incubate 10 min at 22°C.
3. Use up to 5 μL of the mixture for transformation of 50 μL of chemically competent cells or 1-2 μL per 50 μL of electrocompetent cells.

Note:

- The electrotransformation efficiency may be improved by heat inactivation of T4 DNA ligase at 65°C for 10 min or at 70°C for 5 min, and purification of DNA using PCR Purification Kit or by chloroform extraction.
- The overall number of transformants may be increased by extending the reaction time to 1 hour.
- If more than two U of T4 DNA ligase is used in a 20 μ L reaction mixture, it is necessary to purify DNA (by spin column or chloroform extraction) before electrotransformation.

Blunt-end ligation

1. Prepare the following reaction mixture:

Component	20 μL Reaction
Linear vector DNA	20-100 ng
Insert DNA:	1:1 to 5:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2 μL
50% PEG Solution	2 μL
T4 DNA Ligase	5 U
Water, nuclease-free	to 20 μL

2. Incubate for 1 hour at 22°C.
3. Use up to 5 µL of the mixture to transform 50 µL of chemically competent cells. Purify DNA for electrotransformation, using the PCR Purification Kit or by chloroform extraction. Use 1-2 µL of DNA solution 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:

Component	50 µL Reaction
Linear vector DNA	10-50 ng
10X T4 DNA Ligase Buffer	5 µL
T4 DNA Ligase	5 U
Water, nuclease-free	to 50 µL

2. Mix thoroughly, spin briefly and incubate 10 min at 22°C.
3. Use up to 5 µL of the mixture to transform 50 µL of chemically competent cells and 1-2 µL per 50 µL of electrocompetent cells.

Note:

- The electrotransformation efficiency may be improved by heat inactivation of T4 DNA ligase at 65°C for 10 min or at 70°C for 5 min, and purification of DNA, using PCR Purification Kit or by chloroform extraction.
- The overall number of transformants may be increased by extending the reaction time to 1 hour.

Important Notes:

- Polyethylene glycol (PEG) greatly increases the ligation efficiency of blunt-end DNA ligation. The recommended concentration of PEG in the ligation reaction mixture is 5% (w/v).
- Do not exceed the recommended amount of T4 DNA Ligase in the reaction mixture.
- Binding of T4 DNA Ligase to DNA may result in a band shift in agarose gels. To avoid this, incubate samples with Loading Dye & SDS Solution at 65°C for 10 min and chill on ice prior to loading.
- For efficient transformation, the volume of the ligation reaction mixture should not exceed 10% of competent cell volume.

LINKER LIGATION

Double stranded oligonucleotide linkers are often used to generate overhangs not found in the insert. Linkers normally 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:

Component	20 µL Reaction
Linear vector DNA	100-500 ng
Phosphorylated linkers	1-2 µg
10X T4 DNA Ligase Buffer	2 µL
50% PEG Solution	2 µL
T4 DNA Ligase	2 U
Water, nuclease-free	to 20 µL

2. Mix thoroughly, spin briefly and incubate for 1 hour at 22°C.
3. Heat inactivate at 65°C for 10 min or at 70°C for 5 min.

Note:

T4 DNA Ligase is active in PCR and restriction digestion buffers. Therefore, 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 0.5 mM. After inactivation of the T4 DNA Ligase, add the restriction enzyme directly to the reaction mixture and incubate according to the digestion protocol.