T4 DNA Ligase

Technical Manual No. TM0230 **Version 02092007**

I. DESCRIPTION

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA with blunt or cohesive-end termini. The enzyme repairs single-strand nicks in duplex DNA, RNA or DNA-RNA hybrids but has no activity on single-stranded nucleic acids.

II. APPLICATIONS

- Cloning of restriction fragments.
- Joining linkers and adapters to blunt-ended DNA

III. CONTENTS

- T4 DNA Ligase
- 10X T4 DNA Ligase reaction buffer: 500 mM Tris-HCl, 100 mM $MgCl_2$, 5 mM ATP, 100 mM Dithiothreitol, 250 µg/ml BSA, pH7.5 at 25°C

IV. UNIT DEFINITION

One unit is defined as the amount of enzyme required to give 90% ligation of 6 µg Hind III fragments of λ DNA in a total reaction volume of 20 µl in 30 minutes at 16°C in 1X T4 DNA Ligase Reaction Buffer.

V. QUALITY CONTROL

Purified free of contaminating endonucleases and exonucleases. Each lot of T4 DNA Ligase is also tested in a mock cloning assay.

VI. STORAGE BUFFER AND CONCENTRATION

Supplied in 400 U/µl in a 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml BSA, 50% Glycerol, pH7.4 at 25°C.

VII. STORAGE

Store at -20°C. Stable for at least one year if stored properly.

VIII. GENERAL PROTOCOL USING T4 DNA LIGASE

A. Protocol for DNA Insert Ligation into Vector DNA

1. Assemble the following reaction in a sterile micro-centrifuge tube:

Component	Volume
Vector DNA	100 ng
Insert DNA	50 ng
10X Ligation Buffer	2 µl
T4 DNA Ligase	1 µl
Nuclease-free Water to final volume	20 µl

Vortex the tube and spin down in a micro-centrifuge for 3-5 seconds.
2. Incubate the mixture for 2 hours at 16°C.
3. InactivateT4 DNA Ligase by heating the mixture at 65°C for 10 minutes.
4. Use the mixture for transformation.

Note:
1. If the yield of ligation product is insufficient, prolong the reaction time (overnight at 4°C).
2. The resulting ligation reaction mixture can be used directly for bacterial transformation. An excess of ligation mixture with respect to competent cells may decrease the transformation efficiency.
3. Prior to electron-transformation, the ligation mixture should be extracted with chloroform and precipitation with ethanol.

B. Protocol for Self-circularization of Linear DNA

1. Assemble the following reaction in a sterile micro-centrifuge tube:

Component	Volume
Linear DNA	25-50 ng
10X Ligation Buffer	5 µl
T4 DNA Ligase	1-2 µl
Nuclease-free Water to final volume	50 µl

Vortex the tube and spin down in a micro-centrifuge for 3-5 seconds.
2. Incubate the mixture for 2 hours at 16°C.
3. InactivateT4 DNA Ligase by heating the mixture at 65°C for 10 minutes.
4. The resulting reaction mixture can be used directly for transformation.

C. Protocol for Linker Ligation

1. Assemble the following reaction in a sterile micro-centrifuge tube:

Component	Volume
Blunt ended, dephosphorylated DNA	100-500 ng
Phosphorylated linker	1-2 µg
10×Ligation Buffer	2 µl
T4 DNA Ligase	1 µl
Nuclease-free Water to final volume	20 µl

   Vortex the tube and spin down in a micro-centrifuge for 3-5 seconds.
2. Incubate the mixture for 2 hours at 16°C.
3. InactivateT4 DNA Ligase by heating the mixture at 65°C for 10 minutes
4. The resulting ligation products can be digested directly with restriction endonucleases.

Note:
1. Some restriction endonucleases are susceptible to star activity in ligation buffer. Therefore, DNA should be precipitated with isopropanol or ethanol after ligation and dissolved in an appropriate buffer.
2. The recommended linker: DNA fragment molar ratio is >100:1.

IX. ORDER INFORMATION

T4 DNA Ligase, Cat. No. E00016

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