B24102

Baran Biotech

T4 DNA Ligase

50 µL (1 reaction/µL)

Overview

T4 DNA Ligase, derived from the bacteriophage T4, is one of the most widely used ligases in molecular biology. The enzyme catalyzes the formation of phosphodiester bonds between adjacent DNA termini in the presence of the cofactor ATP. During the ligation reaction, T4 DNA Ligase utilizes the energy from ATP to activate the 5' phosphate group and subsequently joins it to the 3' hydroxyl group, thus restoring the continuity of the DNA backbone.

T4 DNA Ligase efficiently seals both sticky (cohesive) and blunt DNA ends, making it an essential tool for applications such as gene cloning, recombinant plasmid construction, and DNA library preparation for next-generation sequencing (NGS).

Enzymatic activity depends on optimal ATP concentration, temperature, and buffer composition. Typical ligation reactions are performed at 16°C, though the conditions can be adjusted depending on the nature of DNA ends and the vector architecture.

Kit Components Item Quantity

Item	Quantity
T4 DNA Ligase (1 reaction/µL)	50 µL
10X T4 DNA Ligase Buffer	0.5 mL
50% PEG Solution	0.3 mL

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% (v/v) glycerol.

10X T4 DNA Ligase Buffer

400 mM Tris-HCl, 100 mM MgCl2, 100 mM DTT, 5 mM ATP (pH 7.8 at 25°C).

50% PEG Solution

50% (w/v) polyethylene glycol 4000.

Source

Escherichia coli cells cloned with the gene 30 of bacteriophage T4.

Unit Definition:

The T4 DNA Ligase provided in this kit is optimized for high-efficiency ligation reactions. Each 1 µL aliquot is standardized to perform one complete ligation reaction, delivering an activity equivalent to 5 Weiss Units (as referenced against Thermo Scientific #EL0014) under standard assay conditions: 1 µg of BamHI-digested DNA fragments in a 20 µL reaction volume at 16 °C for 30 minutes in 1X reaction buffer. The kit contains sufficient enzyme for 50 standardized ligation reactions, eliminating the need for dilution or complex unit conversions.

Protocol

1. Sticky End Ligation

1. Prepare the following reaction mixture:

component	amount
Linear vector DNA	20-100 ng
Insert DNA	1:1 to 10:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2 µ
T4 DNA Ligase	1 µL
Water	to 20 µL

2. Incubate for 1 hour at 16°C, preferably overnight at 4°C

3. Use up to 10 µL of the mixture for chemical competent cells transformation.

2. Blunt-end ligation

1. Prepare the following reaction mixture:

component	amount
Linear vector DNA	20-100 ng
Insert DNA	1:1 to 10:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2 µL
50% PEG Solution	2 µL
T4 DNA Ligase	1 µL
Water	to 20 µL

2. The preferred incubation is overnight at 4°C, alternatively for 1 hour at 16°C if time is limited.

3. Use up to 10 µL of the mixture for chemical competent cells transformation.

Notes:

1. It is recommended to purify DNA for electro transformation. Use 1-2 µL of DNA solution per 50 µL of electrocompetent cells.

2. The optimal insert/vector ratio is 3:1, but this can be adjusted from 1:1 up to 10:1 depending on your ligation strategy and the necessity to minimize vector self-ligation.

3. The quality, purity, and accurate concentration of DNA fragments are critical for a successful ligation; careful assessment of these parameters is strongly recommended.

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

5. T4 DNA Ligase is inactivated by heating at 65°C for 10 min or at 70°C for 5 min.