

# E. coli DNA ligase

### Introduction

*E. coli* DNA ligase is a recombinant enzyme purified from Escherichia coli strains, carrying the gene of *E. coli* DNA ligase. *E. coli* DNA ligase catalyzes the formation of phosphodiester bonds between the 5' phosphate and the 3' hydroxyl of adjacent duplex DNA strands, with very low activity for blunt-ended DNA. The enzyme is most effective in Tris-HCl buffer at pH 7.5-8.0 and requires NAD<sup>+</sup> as a cofactor. This enzyme is mainly employed for nick sealing and cohesive DNA end ligation. *E. coli* DNA ligase remains active within a temperature range of 4-37°C.

## **Components and Storage**

Size Components	K3154-200 U	K3154-1000 U	Storage
E. coli DNA ligase (10 U/μL)	20 µL	100 µL	<b>-20</b> ℃
10 × E. coli DNA ligase	200 ці	1 mL	<b>-20</b> °C
Reaction Buffer	200 μΕ		
Shipping: Dry Ice	e Shelf life: 12 months		

# Protocol



- APERBIO
- 1. Prepare the reaction system on ice according to the table below:

Components	20 µL Reaction	Final Concentration
dsDNA	ΧμL	up to 0.25 µg/µL
10 × E. coli DNA ligase Reaction Buffer	2 µL	810 1×
E. coli DNA Ligase (10 U/µL)	1 µL	0.5 U/µL
Nuclease-free Water	Add to 20 µL	1

- 2. After setting up the reaction system according to the table above, gently mix (using a pipette for gentle pipetting or low-speed vortexing) and then centrifuge.
- **3.** Incubate at  $16^{\circ}$ C for 30 minutes.
- 4. Incubate at  $65^{\circ}$ C for 20 minutes to terminate the reaction.

#### Product properties

- Enzyme activity unit: 1 unit is defined as the amount of enzyme required to ligate 50% of λ DNA fragments digested by HindIII (with a 5' DNA end concentration of 0.12 µM, or 300 µg/mL) in a total reaction volume of 20 µL at 16°C for 30 minutes in 1X E.coli DNA ligase Reaction Buffer.
- Storage solution: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/mL Recombinant Albumin, 50% Glycerol (pH 7.4 @ 25℃).
- 3. Inactivation conditions: Incubate at 65 °C for 20 minutes.

#### Note

**1.** The 10 × *E. coli* DNA ligase Reaction Buffer contains NAD<sup>+</sup> (nicotinamide adenine dinucleotide), and it is recommended to store it at -80 °C for long-term storage to extend its half-life.

APENBIO

APERBIO

- 2. *E. coli* DNA Ligase can only ligate DNA fragments with cohesive ends and has very low efficiency for ligating blunt-ended fragments. To ligate blunt-ended DNA, you may add polyethylene glycol (PEG) and high concentrations of monovalent cations.
- 3. The experiments should be performed on ice to avoid prolonged exposure at room temperature, which can affect its activity.
- 4. For research use only. Not to be used in clinical diagnostic or clinical trials.

APETBIO

