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T4 DNA Ligase for NGS

Cat. No. : CW2701S (1500 U)
CW2701M (7500 U)
Storage Condition: -20°C, transport with dry ice.

Components

Component	CW2701S 1500 U	CW2701M 7500 U
T4 DNA Ligase，15 U/μL	100 μL	500 μL
4×T4 DNA Ligase Buffer	600 μL	2×1.5 mL

Introduction

T4 DNA Ligase is isolated and purified from Escherichia coli expressing T4 DNA Ligase gene after induced expression, and can catalyze the phosphodiester bond between the 5 'phosphate group and 3' hydroxyl group of adjacent DNA chains. This enzyme can catalyze the ligation of blunt end or sticky end DNA, repair single-strand cuts in double-stranded DNA, RNA, and single-strand DNA/RNA hybridization, but has no activity for single-stranded nucleotides.

Activity definition

1U refers to the amount of enzyme required to convert 1 nmol [32PPi] to the absorbable form of Norit at 37°C in 20 minutes in an ATP-PPi exchange reaction, equivalent to about 200 sticky end-ligating units.

Application

It is mainly used to ligate Adapter during library preparation in NGS.

Protocol

It is recommended to use Cowin's Adapter, or you can also use NEB's or Illumina's Adapter. For specific ligate methods, refer to the product manuals of each company. The following is how to ligate using Cowin's adapter:

1. Add the following reagents directly to the DNA end repair reaction solution:

Reagent name	Volume
4×T4 DNA ligase Buffer	25 μL
T4 DNA ligase, 15 U/μL	5 μL
Adapter	5 μL
ddH_2O	To 50 μL

   The total volume of solution in the tube is 100 μL.

   Note: If the starting sample is less than 100 ng, dilute the Adapter with deionized water 10 times to 1.5 μM before use.

2. Pipette the reagent to mix, and then centrifuged briefly to collect the solution at the bottom of the tube.
3. Bath at 23°C for 20 minutes.

   Note: If this operation is performed using a PCR instrument, please close the hot lid.
4. Proceed to the subsequent steps, such as size selection of DNA fragments or purification of DNA fragments.