Product Manual

Product Name	T5 Exonuclease
Source	Recombinant expression in Escherichia coli
Catalog Number	CSB-DEM047
Physical Form	Liquid
Enzyme Activity	10U/μL
Storage Conditions	-20 ±5°C
Storage Buffer	50mM Tris-HCl (pH 7.5), 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.1% (v/v) Triton X-100, 50% (v/v) Glycerol
Activity Definition	At 37°C, in a 50μL reaction volume, the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotides from double-stranded DNA within 30 minutes is defined as 1 unit (U).
Quality Control	c
Shelf Life	24 months

Product Description

T5 Exonuclease is a nucleic acid exonuclease that degrades double-stranded or single-stranded DNA in the 5'→3' direction. It can initiate digestion from the 5' end of single-stranded or double-stranded DNA and also from gaps or nicks in linear or circular double-stranded DNA. T5 Exonuclease cannot degrade supercoiled double-stranded DNA, and its activity on single-stranded DNA can be inhibited by reducing the Mg2+ concentration in the reaction buffer to below 1mM. Based on these characteristics, T5 Exonuclease is commonly used in Gibson Assembly.

Gibson Assembly is a technique for efficiently connecting multiple overlapping sequence fragments under isothermal conditions. The basic principle can be summarized in three steps: (1) T5 Exonuclease digests the DNA fragments from the 5' end, generating complementary single-stranded 3' overhangs that facilitate annealing of

complementary ends; (2) DNA polymerase fills in the gaps created by annealing; (3) DNA ligase connects the assembled fragments at the nicks, resulting in a complete double-stranded DNA molecule.

Inactivation or Inhibition of T5 Exonuclease:

Adding EDTA to a final concentration of at least 11mM or using DNA loading buffer containing SDS (final concentration of SDS is 0.08%) can inactivate T5 Exonuclease.

Product components

Component No.	Component Name	Specifications		
1	10×Reaction Buffer	0.25mL	0.5mL	2.5mL
2	T5 Exonuclease	500U	1000U	5000U

Operating instructions

1. Set up the reaction system in an ice bath

Component	Volume
DNA	1 μg
10×Reaction Buffer	5 μL
T5 Exonuclease(10U/μL)	1 μL
ddH2O	up to 50 μL

Note:

T5 Exonuclease should be added last and mixed thoroughly before addition. It is recommended to store the enzyme in an icebox or on ice. Gently mix the reaction system and centrifuge at low speed to collect the liquid at the bottom of the tube.

2. Gently mix the reaction system and centrifuge at low speed to collect the liquid at the bottom of the tube

3. **Reaction Conditions：** Incubate at 37ºC for 10-30 minutes.

4. **Termination of Reaction:** Immediately cool the reaction and add EDTA to a final concentration of 11mM to stop the reaction.

Note:

1. T5 Exonuclease is a nucleic acid exonuclease that exhibits selective activity towards different DNA substrates. Therefore, when digesting specific substrates, it is important to control the enzyme concentration and reaction time appropriately.

2. The optimal reaction temperature for T5 Exonuclease is 37°C, but it also exhibits some activity at 50°C, making it suitable for Gibson Assembly.

3. T5 Exonuclease also exhibits activity in regular PCR buffers.