T5 Exonuclease

REF: EG24208S

Storage Condition

-20℃

Components

Component	Amount
T5 Exonuclease (10 U/μl)	100 μl
10× T5 Reaction Buffer	1 ml

Description

T5 Exonuclease is a nucleic acid exonuclease that digests DNA from the 5' end in the 5'→3' direction for both double-stranded and single-stranded DNA. It is capable of digesting DNA at nicks or gaps in linear or circular double-stranded DNA,however, it cannot digest supercoiled DNA. In addition, when the Mg^{2} concentration in the solution is below 1 mM, the activity of T5 Exonuclease towards single-stranded DNA is inhibited. Consequently, T5 Exonuclease is particularly suitable for degrading linearized and nicked plasmids, removing non-circular DNA from ligation products, seamless cloning (Gibson Assembly), and other applications.

Definition of Activity Unit

One unit (U) of activity is defined as the amount of enzyme required to cause a change in A260 of 0.00032 per minute, using salmon sperm DNA as the substrate, under the conditions of 37℃ and 1× T5 Reaction Buffer.

Applications

1. Degradation of linear single-stranded, double-stranded DNA, or nicked plasmid DNA.
2. Remove the incomplete ligation products from the circularized double-stranded DNA.
3. To obtain high-purity supercoiled plasmid DNA, it is necessary to remove the denatured plasmid DNA produced during the alkaline lysis method for plasmid extraction, and degrade the linear and nicked plasmid DNA.
4. Improve the transfection efficiency of the small-scale cDNA library.
5. Commonly used in Gibson Assembly.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 50 μl reaction containing 1 μg of supercoiled plasmid and 10 U of T5 Exonuclease incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Residual Host DNA

The product was tested by TaqMan qPCR with primers specific for the E.coli 16S rDNA , and the results show that the E.coli genome residues less than 1 copy.

Protocol

Set up the following reaction on ice:

Reagent	Amount
DNA	1 μg
10× T5 Reaction Buffer	5 μl
T5 Exonuclease (10 U/μl)	1 μl
Nuclease-Free Water	up to 50 μl

Reaction conditions: 37℃ 30 min.

Heat Inactivation: 80℃ 20 min，Add EDTA to a final concentration of at least 11 mM, or add DNA Loading containing SDS (final concentration of SDS should be 0.08%).

Notice

1. T5 Exonuclease is a non-specific DNA exonuclease that has different reaction rates for different types of DNA. When performing the reaction, it is important to select appropriate enzyme amounts and reaction times.
2. T5 Exonuclease exhibits optimal reaction activity at 37℃ and retains some activity at 50℃ , making it suitable for Gibson assembly.
3. For your safety and health, please wear a lab coat, disposable gloves, and a mask while conducting the experiment.

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