T5 Exonuclease

T750876

Store at -20℃

Introduction:

T5 Exonuclease is a specific exonuclease that degrades double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) in the 5' to 3' direction. It is able to initiate nucleotide removal from the 5' termini or at gaps and nicks of linear or circular dsDNA. However, it does not degrade supercoiled dsDNA. T5 Exonuclease also has ssDNA endonuclease activity, which can be inhibited by reducing the Mg^{2+} concentration to less than 1mM in the reaction mixture. T5 Exonuclease is widely used in Gibson Assembly.

Gibson assembly is a simple and rapid approach for efficient assembly of DNA fragments with overlapped regions in a correct order. The reaction is carried out under isothermal conditions by three enzymes: (1) T5 exonuclease degrades DNA fragment at 5' terminus and generates complementary overhangs at 3' end; (2) DNA polymerase fills the gaps of the annealed single strand regions; (3) DNA ligase seals the nicks of the annealed and filled-in gaps, producing intact dsDNA (plasmid).

Applications:

Gibson assembly; degradation of linear DNA and nicked plasmid DNA; removal of incomplete ligation products from ligated circular dsDNA; degradation of linear and nicked plasmid DNA to obtain high-purity supercoiled plasmid DNA; removal of the denatured DNA from alkaline-based plasmid purification methods; improve the transfection efficiency of mini extracted plasmid cDNA library.

Source:

Expressed and purified from E. coli transformed with the T5 phage D15 plasmid.

Activity definition:

One unit is defined as the amount of enzyme required to produce 1nmol of acid soluble deoxyribonucleotide from double-stranded DNA in 30 minutes at 37℃ in a total reaction volume of 50μl.

Purity:

Free from RNase, phosphatase, DNA endonuclease and other SNA exonucleases except T5 Exonuclease.

Enzyme storage buffer:

50mM Tris-HCl (pH7.5, 25℃), 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.1% (v/v) Triton X-100, 50% (v/v) Glycerol.

10X Reaction Buffer:

200mM Tris-acetate (pH7.9, 25℃), 500mM Potassium Acetate, 100mM Magnesium Acetate, 10mM DTT

Inactivation or inhibition:

T5 Exonuclease can be inactivated by adding EDTA to a final concentration of at least 11mM or by adding DNA loading buffer containing SDS to a final SDS concentration of 0.08%.

Usage method:

Set up the reaction mixture on ice as follows:

Reagent	Volume	Final
DNA	X μL	0.02 μg/μL
Nuclease-free Water	(44-x) μL	-
10X Reaction buffer	5μL	1X
T5 Exonuclease	1μL	0.2U/μL
Total Volume	50μL	-

Note: T5 Exonuclease should be added at last, after mixing well all other contents. Keep T5 Exonuclease on ice during the reaction assembling.

1. Mix the reaction well and have a quick spin to allow all the liquid accumulate at the bottom of the tube.
2. Incubate at 37℃ for 10-30min.
3. Immediately transfer the reaction on ice and terminate the reaction by adding EDTA to a final concentration of 11mM.

Matters needing attention:

1. T5 Exonuclease is a selective exonuclease that displays different activities to different DNA substrates. Therefore, the amount of enzyme and the incubation time should be optimized for different DNA substrates.
2. The optimal reaction temperature of T5 Exonuclease is 37℃. But it is also active at 50°C and therefore can be used for Gibson assembly.
3. T5 Exonuclease is compatible with regular PCR buffers.
4. This product is for R&D only. Not for drug, household, or other uses.
5. For your safety and health, please wear lab coat and disposable gloves during the operation.