T5 Exonuclease (RNase-free)

Introduce

T5 Exonuclease is a nucleic acid exonuclease that degrades double- or single-stranded DNA in a 5' →3' direction. Degradation can begin either from the 5' end of double- or single-stranded DNA, or from gaps or nicks in linear or cyclic double-stranded DNA, but not from superhelix double-stranded DNA. T5 Exonuclease is often used for Gibson Assembly; Degradation of linear single-stranded, double-stranded DNA or absent plasmid DNA; Removal of incomplete ligation products from the linked cyclic double-stranded DNA; Degradation of linear and absent plasmid DNA to obtain high-purity supercoiled plasmid DNA; Removal of metamorphic DNA produced during plasmid extraction by alkali cleavage; Improve transfection efficiency of small extract plasmid cDNA libraries.

Composition and storage conditions

Components / Size	1000 U	5000 U	10000 U
T5 Exonuclease (RNase-free)	0.1 ml	0.5 ml	1 ml
10X T5 Exonuclease Reaction Buffer	1 ml	1 ml x5	1ml x10
Store at -20 °C.			

Experimental operation

1. Configure the reaction system in the ice bath according to the following table:

Reagent	Volume	Final Concentration
DNA	X µl	1 µg
10X Reaction buffer	5µl	1X
T5 Exonuclease	1µl	10 U
Nuclease-free H2O	to 50µl	-

2. Gently mix the reaction system up and down with a pipette and centrifuge quickly.
3. Incubate at 37 °C for 10~30min.
4. Stop the reaction with 6X Purple Gel Loading Dye or adding EDTA to at least 11 mM.
5. The digested products are analyzed by agarose gel or polypropylene gel electrophoresis.

Note: T5 Exonuclease should be added to the reaction system last, and the mixing reaction system should be paid attention to before joining; Store in an ice box or on an ice bath.

Note

1. Definition of Enzyme Activity Units: The amount of enzyme required to catalyze the production of 1 nmol of DNA from a double-stranded DNA substrate within 30 minutes in a 50 μL reaction system at 37 °C.

2. Liquid components in T5 nucleic acid-free exonuclease: 50 mM Tris-HCl (pH 7.5, 25 °C), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% TritonX-100, 50% glycerol.

3. Buffer components in the 10X T5 Exonuclease Reaction Buffer：500 mM Potassium Acetate，200 mM Tris-acetate (pH 7.9, 25°C)，100 mM Magnesium Acetate，10 mM DTT.

4. T5 Exonuclease (RNase-free) quality and experimental validation:

   I. Protease purity > 95%.

   II. Verification of activity:

   - 10U of T5 Exonuclease and 200ng of 300 base RNA substrate reacted at 37 °C for 4 h with no change in the electrophoresis band of RNA.
   - 50U of T5 Exonuclease and 1 μg of supercoiled PhiX174 DNA reacted at 37 °C for 4 h without alteration of the electrophoretic band of the DNA.

5. T5 Exonuclease inactivation conditions: T5 Exonuclease can be inactivated by adding a final concentration greater than 11 mM EDTA to terminate the reaction, or a DNA loading buffer containing SDS (0.08% final concentration of SDS).