Certificate of Analysis & Data Sheet

Catalog Number: 230-06004

RayBiotech
Empowering your proteomics

Recombinant T5 Exonuclease

Source

Species	*Enterobacteria phage T5*
Gene Symbols	D5
Synonyms	5'-3' exonuclease, exodeoxyribonuclease, EC:3.1.11.3.

Preparation

Expression Host	*E.coli*
Tag or Conjugation	N-terminal His-tag
Purification	His-tag affinity purification by immobilized metal ion affinity chromatography (IMAC)
Purity	>95% as determined by SDS-PAGE under reducing conditions
Endotoxin	<0.5 EU per μg of the protein as determined by the LAL method
Molecular Weight	~38 kDa

Specifications

Form	Liquid
Formulation	Filtered solution in 50 mM Tris-HCl (pH 8.0, 25 ℃), 125 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.2% (v/v) Triton X-100, and 50% glycerol.
SDS-PAGE Image	See **Fig. 1** below
Activity	12.5 U/μl. One unit is the amount of enzyme required to change A260 value by 0.00032 per minute at 37 °C.
Applications	T5 exonuclease is double-stranded DNA (dsDNA) specific exonuclease and single-stranded DNA endonuclease. It can cleave the nicked double-stranded DNA from 5' end to 3' end (**Fig. 2**). However, the enzyme can not cleave the close supercoiled double-stranded DNA (**Fig. 3**). It can remove incomplete ligation products from the ligated circular dsDNA and widely used for Gibson assembly.

User Instruction

1) Set-up the cleavage reaction on ice according to following chart. Add T5 exonuclease lastly.

	Reagents	Volume (50 μl)
1	DNA sample (up to 1 μg)	x μl
2	10× Cleavage Buffer	5 μl
3	Sterile DNase and RNase-free water	44 - x μl
4	T5 Exonuclease (12.5 units/μl)	1 μl
	Final Volume	50 μl

2) Gently mix (don't vortex the samples) and spin down the reaction briefly. Incubate at 37°C for 30 minutes.

3) Stop reaction on ice through adding 11 mM EDTA or DNA Loading Buffer containing 0.08% SDS. Check DNA cleavage by agarose gel electrophoresis.

4) Take 10 μl reaction and mix with 2 μl 6x DNA Loading Buffer. Analyze DNA cleavage by 2% agarose gel electrophoresis.

Data

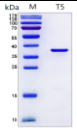

Fig. 1 SDS-PAGE analysis of purified T5 product. *M* , protein ladder (kDa).

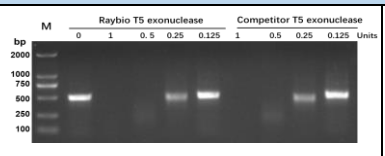

Fig. 2 PCR product dsDNA cleavage. *M* , DNA ladder (bp).

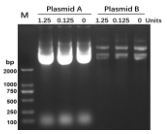

Fig. 3 Supercoiled high copy plasmid (A) and low copy plasmid (B) DNA cleavage

Shipping

The product is shipped with ice packs, including one vial of 10x Cleavage Buffer. Upon arrival, immediately store product at the temperature recommended below.

Storage/Stability

Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

For long term storage, it is recommended to store at -20 °C in appropriate aliquots.

Generally, the shelf life is up to 6 months from the date of receipt at -20 °C under sterile condition.