Intact Genomics®

T5 Exonuclease

Catalog #	3372	3374
Package Size	1,000 units	5,000 units
Volume	100 µl	500 µl
Concentration	10 units/µl	

Description

T5 Exonuclease is a highly efficient 5´→3´ exonuclease for either ssDNA or dsDNA. It also has ssDNA specific endonuclease activity in the presence of magnesium ions. However, the enzyme does not degrade supercoiled dsDNA [1]. The mode of action of T5 Exonuclease *in vivo* may be analogous to that of the 5´→3´ exonuclease activity of *E. coli* DNA Polymerase I [1, 2].

Protein Purity

The physical purity of this enzyme is ≥98% as assessed by SDS-PAGE with Coomassie® blue staining (see figure below).

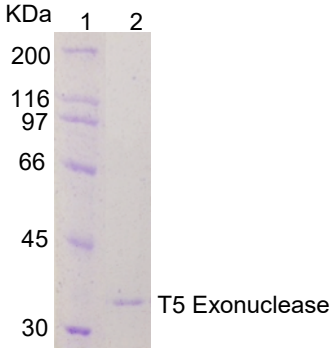

Figure: Lane 1. Protein Marker
Lane 2. T5 Exonuclease

Product Source

E. coli strain expressing the T5 phage D15 gene.

Applications

- Plasmid mutagenesis methods.
- Oligonucleotide site-directed mutagenesis.
- Generation of plasmid-sequencing templates.
- Generation of 3'-overhang for improved cloning procedures.
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Product Includes

1) T5 Exonuclease
2) 10x T5 Exonuclease Reaction Buffer

Storage Temperature

–20 °C

Storage Buffer

50 mM Tris-HCl
50 mM KCl
1 mM DTT
0.1 mM EDTA
50% Glycerol
pH 7.5 @ 25 ºC

10x T5 Exonuclease Reaction Buffer

330 mM Tris-acetate (pH 7.5)
660 mM potassium acetate
100 mM magnesium acetate
5.0 mM DTT

Unit Definition

One unit of T5 Exonuclease catalyzes the release of 1 nmol of acid-soluble nucleotides from double-stranded calf thymus DNA in 30 minutes at 37 °C under standard assay conditions.

Quality Control

T5 Exonuclease is free from detectable RNase or contaminating DNA endonuclease activities.

Protocol

1. Set-up the reaction as follows:

DNA	x µl (up to 1 µg)
10X Buffer	5.0 µl
T5 Exonuclease	1.0 µl
H_2O up to	50.0 µl

2. Incubate at 37 °C for 15-30 minutes.
3. Add 10 mM EDTA to stop the reaction.
4. Clean-up treated samples by column purification or phenol/chloroform extraction followed by ethanol precipitation.

References

1. Sayers, J.R. and Eckstein, F. (1990) *J. Biol. Chem.* 265, 18311.
2. Sayers, J.R. et al. (1991). Nucleic Acids Res. 19, 4127-4132.

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