

T5 Exonuclease



M0363S 001160918091

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1,000 units **10,000 U/ml** **Lot: 0011609**

RECOMBINANT **Store at -20°C** **Exp: 9/18**

Description: T5 Exonuclease degrades DNA in the 5' to 3' direction (1). T5 Exonuclease is able to initiate nucleotide removal from the 5' termini or at gaps and nicks of linear or circular dsDNA (1). However, the enzyme does not degrade supercoiled dsDNA (2). The ssDNA endonuclease activity has been shown to be suppressed by lowering the concentration of Mg²⁺ to less than 1 mM (1). This protein is the product of T5 phage D15 gene (3).

Source: An *E. coli* strain that carries a plasmid with the T5 phage D15 gene.

Supplied in: 100 mM NaCl, 50 mM Tris-HCl (pH 7.5 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

Applications:

- Degradation of linear ssDNA, dsDNA or nicked plasmid DNA while maintaining supercoiled plasmid DNA.
- Remove denatured DNA isolated during alkaline lysis-based plasmid purification procedure (5).
- Enhance the transfection efficiency of miniprep from plasmid cDNA libraries (6).

Reagents Supplied with Enzyme:

10X NEBuffer 4

Reaction Conditions:

1X NEBuffer 4.
Incubate at 37°C.

1X NEBuffer 4:

50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 4 with 0.15 mM sonicated duplex [³H]-DNA.

Unit Assay Conditions: 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9) and 0.15 mM sonicated duplex [³H]-DNA.

Heat Inactivation:

No

Quality Control Assays

Endonuclease Activity: Endonuclease Activity: Incubation of a 50 µl reaction containing 30 units of T5 Exonuclease with 1 µg of supercoiled pUC19

DNA in NEBuffer 4 for 16 hours at 37°C resulted in < 10% loss in supercoiled pUC19 DNA as determined by agarose gel electrophoresis.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

References:

1. Sayers, J.R. et al. (1991) *Nucleic Acids Res.* 19, 4127-4132.
2. Sayers, J.R. et al. (1990) *J. of Biol. Chem.* 265 18311-18317.
3. Kaliman, A.V. et al. (1986) *FEBS.* 195, 61-64.
4. Frenkel, G.D. et al. (1971) *J. of Biol. Chem.* 246, 4839-4847.
5. Sayers, J.R. et al. (1996) *Analytical Biochem.* 241, 186-189.
6. Kiss-Toth, E. et al. (2001) *Analytical Biochem.* 288, 230-232.

CERTIFICATE OF ANALYSIS

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