



PRODUCT INFORMATION

Endonuclease V, *T.maritima*

Pub. No. MAN0011995

Rev. Date 13 June 2016 (B.00)

#EN0141

Lot: _____ Expiry Date: _____

Components	#EN0141 250 U
Concentration	5 U/μL
10X Reaction Buffer	0.3 mL

Store at -20 °C

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Description

Endonuclease V, *T.maritima* (Endo V), is a 3'-endonuclease involved in DNA repair which initiates removal of deaminated bases from damaged DNA, including uracil, hypoxanthine and xanthine. Endonuclease V is also active toward abasic sites and urea sites, base pair mismatches, flap and pseudo Y structures, and small insertions/deletions in DNA molecules. The cleavage site generated by Endonuclease V is at the second phosphodiester bond 3' to a lesion. When the enzyme is in excess, the primary nicked products experience a second nicking event on the complementary strand, leading to a doublestranded break. At low concentrations, however, Endonuclease V first nicks a DNA strand at the lesions located closer to the 5'-end of DNA molecule. Single-stranded DNA is cleaved with much lower efficiency. Mg²⁺ or Mn²⁺ ions are required for enzyme activity (1, 2, 3).

Applications

- High-throughput methods for mutation research (3, 4).
- Studies in mutagenesis and DNA repair.
- Mismatch cleavage.
- Genotyping.

Source

E.coli with a cloned *nfi* gene of *Thermotoga maritima*.

Molecular Weight

25 kDa monomer.

Definition of Activity Unit

One unit of the enzyme relaxes one μg of depurinated, supercoiled plasmid DNA in 30 min at 65 °C.

Enzyme activity is assayed in the following mixture:

25 mM HEPES-NaOH (pH 7.4), 5 mM MgCl_2 , 5 mM DTT, 2% (v/v) glycerol, 2 μg of depurinated pUC19 DNA.

Storage Buffer

The enzyme is supplied in: 20 mM HEPES-NaOH (pH 7.4), 5 mM DTT, 50 mM NaCl, 0.1% (v/v) Triton X-100 and 50% (v/v) glycerol.

10X Reaction Buffer

250 mM HEPES-NaOH (pH 7.4 at 25 °C), 50 mM MgCl_2 , 50 mM DTT, 20% (v/v) glycerol.

Inhibition and Inactivation

- Inhibitors: no specific inhibitors have been described.
- Inactivated by heating in boiling water bath for 10 min, preferably in the presence of EDTA.

CERTIFICATE OF ANALYSIS

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded radiolabeled oligonucleotides after incubation with Endonuclease V.

Ribonuclease Assay

No detectable degradation was observed after incubation of $[^3\text{H}]$ -RNA with Endonuclease V.

Quality authorized by:  Jurgita Zilinskiene

References

1. Huang, J., et al., Multiple cleavage activities of endonuclease V from *Thermotoga maritima*: recognition and strand nicking mechanism, *Biochemistry*, 40(30), 8738-8748, 2001.
2. Hitchcock, T.M., et al., Cleavage of deoxyoxanosine-containing oligodeoxyribonucleotides by bacterial endonuclease V, *Nucleic Acids Res.*, 32(13), 4071-4080, 2004.
3. Pincas, H., et al., High sensitivity EndoV mutation scanning through real-time ligase proofreading, *Nucleic Acids Res*, 32, 148, 2004.
4. Huang, J., et al., An endonuclease/ligase based mutation scanning method especially suited for analysis of neoplastic tissue, *Oncogene*, 21, 1909-1921, 2002.

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