Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Endonuclease V, *T.maritima*

#EN0141 250u (5u/µl)

Lot: **Expiry Date:**

Concentration: 5u/µl

Supplied with: 0.3ml of 10X Reaction Buffer

Store at -20°C

In total 2 vials.

ISO 9001 ISO 14001
www.fermentas.com

Description

The Endonuclease V, *T.maritima*, 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 double-stranded 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. Mg^{2+} or Mn^{2+} ions are required for enzyme activity (1, 2, 3).

Source

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

Definition of Activity Unit

One unit of the enzyme converts one µg of supercoiled depurinized plasmid DNA into other topological states in 30min at 65°C.

Activity Assay

25mM Na-HEPES (pH 7.4), 5mM $MgCl_2$, 5mM DTT, 2% (v/v) glycerol, 2µg of partially depurinated pBR322 DNA.

Storage Buffer

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

10X Reaction Buffer

250mM Na-HEPES (pH 7.4), 50mM $MgCl_2$, 50mM DTT, 20% (v/v) glycerol

Applications

- High-throughput methods for mutation research (3, 4).
- Studies in mutagenesis and DNA repair.
- Mismatch cleavage.
- Genotyping.
- Error correction during the synthesis of long polynucleotide sequences (5).

Inhibition and Inactivation

Inactivated by heating in boiling water bath for 10min, preferably in the presence of EDTA, or by phenol/chloroform extraction.

QUALITY CONTROL ASSAY DATA

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 units of Endonuclease V with 1µg of sonicated *E.coli* [^{3}H]-DNA in 50µl of buffer (25mM Na-HEPES (pH 7.4), 5mM $MgCl_2$, 5mM DTT, 2% glycerol) for 4 hours at 37°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 units of Endonuclease V with 1µg of [^{3}H]-RNA in 50µl of buffer (25mM Na-HEPES (pH 7.4), 5mM $MgCl_2$, 5mM DTT, 2% glycerol) for 4 hours at 37°C.

Quality authorized by:  Jurgita Zilinskiene

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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(19), 148, 2004.
4. Huang, J., et al., An endonuclease/ligase based mutation scanning method especially suited for analysis of neoplastic tissue, Oncogene, 21(12), 1909-1921, 2002.
5. M. Fuhrmann, et al., Removal of mismatched bases from synthetic genes by enzymatic mismatch cleavage, Nucleic Acids Res, 33, e58, 2005.

Notice

Use of this enzyme in certain applications may be covered by patents and may require a license.

Related Products

• *Taq* DNA Polymerase	#EP0401, #EP0403, #EP0402, #EP0404 #EP0281, #EP0283, #EP0282, #EP0284 #EP0071, #EP0072
• dNTP Mix, 25mM each	#R1121, #R1122
• dNTP Mix, 10mM each	#R0191, #R0192, #R0193
• dNTP Mix, 2mM each	#R0241, #R0242
• dUTP	#R0133
• dITP	#R1191
• Water, nuclease-free	#R0581 #R0582

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

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