Endonuclease IV (Tth)

Catalogue number: MB43101, 500 U

Description

Endonuclease IV (Tth) is a Class II thermostable Apurinic/aPyrimidinic (AP) DNA endonuclease from *Thermus thermophilus*. This an enzyme is involved in the DNA Base Excision Repair (BER) pathway, that catalyses the cleavage of DNA phosphodiester backbone at AP sites via hydrolysis, leaving a 1 nucleotide gap with 3'-hydroxyl and 5' deoxyribose phosphate (dRP) termini. AP sites are locations in DNA that neither contain a purine nor a pyrimidine base, which occur either spontaneously or due to DNA damage. Endonuclease IV (Tth) displays activity in both ssDNA and dsDNA. The enzyme also displays 3'-diesterase activity.

Storage conditions

Endonuclease IV (Tth) should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit of enzyme activity is defined as the amount of enzyme required to cleave 1 pmol of a 60-mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µL in 1 hour at 65 °C. An AP site is created by treating 10 pmol of a 60-mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37 °C.

Enzyme concentration: 10 U/ µL

Inactivation

Endonuclease IV (Tth) is highly resistant to heat inactivation. Thus, alternative protocols should be considered when requiring removing the enzyme from reactions, such as DNA silica column purification or phenol/chloroform extraction.

System components and Reaction conditions

Endonuclease IV (Tth) is provided with a dedicated and highly optimized NZYtech reaction buffer and displays an optimum temperature of 65 °C.

Quality Control Assays

Purity

Endonuclease IV (Tth) is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYtech, Cat. No. MB15201).

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 10 U of Endonuclease IV (Tth) for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of Endonuclease IV (Tth) for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

Endonuclease IV (Tth) is assayed in a reaction containing 1 pmol of a 60-mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µl in 1 hour at 65°C.

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For research use only.

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