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ProductInformation

Endonuclease III
from *E. coli*, recombinant

Product Number **E 0526**
Storage Temperature 0 to –20 °C

Synonyms: Endo III; Nth protein

Product Description
Endonuclease III (Endo III) is an *E. coli* recombinant protein overexpressed in *E. coli*. It contains 211 amino acids with a molecular weight of approximately 23 kDa. The enzyme is purified as the iron bound form, which has a light green color.

Endonuclease III from *E. coli*, the product of the *nth* gene, is an iron-sulfur containing DNA repair enzyme.[1,2] Endo III possesses DNA glycosylase activity with a broad substrate specificity for mutated pyrimidine derivatives, especially thymine glycol (Tg), but also for 5-hydroxycytosine and 5,6-dihydrothymine.[3] In addition it has an apurinic/apyrimidinic (AP) lyase activity, which cleaves the DNA backbone by β elimination.[4] Endo III modified-base substrate specificity overlaps the substrate specificity of endonuclease VIII (endo VIII, nei protein).[5] This cross reactivity is manifested in *E. coli* mutants. While *E. coli nth* and *nei* mutants are not sensitive or slightly more sensitive to ionizing radiation and hydrogen peroxides than wild type, the *nei-nth* double mutant is hypersensitive to oxidative stress.[2,3]

Endo III protects *E. coli* cells from radiation damage caused by X-ray and UV irradiation.[5,6] When overexpressed, it can also protect *E. coli* cells from alkylation defects caused by methyl methanesulfonate.[7]

The product is supplied as a solution in 50% glycerol containing 20 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, and 1 mM DTT.

Purity: minimum 90% (SDS-PAGE)

Specific Activity: minimum 10,000 units per mg protein

Unit Definition: The amount of protein that cleaves 50% of 0.5 pmole of double stranded oligonucleotide substrate containing 5,6-dihydrothymine (DHT) in 15 minutes at pH 7.6 at 30 °C.

Precautions and Disclaimer
This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on wet ice and storage at 0 to –20 °C is recommended.

Procedure
Reagents & Supplies Required but not Supplied

- ^{32}P labeled Endonuclease VIII and III substrate double stranded oligonucleotide: Prepare the substrate according the product information sheet for the Endonuclease VIII & III Substrate Set, (Product No. E 7651).
- 10X reaction buffer: 500 mM Tris-HCl, pH 7.6, 20 mM EDTA, and 500 mM KCl
- Enzyme dilution buffer: 20 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 1 mM DTT, 10% (v/v) glycerol, and 100 mM NaCl
- Stop solution: 90% formamide, 20 mM EDTA, 0.1% (w/v) bromophenol blue, and 0.1% (w/v) xylene cyanole FF
- 20% denaturing gel
- TBE Running buffer: 89 mM Tris base, 2 mM EDTA, and 89 mM Boric acid, pH 8.0
- X-ray film and developing machine

Principle of Assay
The endonuclease III activity assay is based on its glycosylase activity that recognizes and removes the mutated base 5,6-DHT, which is followed by its lyase activity that cleaves the AP (apurinic/apyrimidinic) strand of the double stranded DNA. The substrate used for assaying the activity of endonuclease III is a radiolabeled double stranded 33 oligonucleotide containing DHT at the 16th base of the labeled strand (Product No. E 7651, Endonuclease VIII and III Substrate Set). In the reaction, endonuclease III first removes the 5,6-DHT and then cleaves the mutated strand at the apyrimidinic site. Denaturation of the

double stranded oligonucleotides and separation on a denatured polyacrylamide gel produces two labeled bands: a 33 bp oligonucleotide band (residual uncleaved substrate) and a 15 bp nucleotide band (the cleavage product).

Assay

1. Prepare 20% denaturing gel containing 7 M urea, assemble the electrophoresis apparatus, and add running buffer.[8]
2. Prepare reaction mix for 10 reactions:

Component	Volume per 10 reactions
10X Reaction Buffer	10 μl
^{32}P labeled substrate	2 μl (~5 pmole)
Distilled water	68 μl

3. Dilute the endo III enzyme to 25, 50, and 100 μg/ml with enzyme dilution buffer.
4. Dispense 8 μl of reaction mix into each tube
5. Start each reaction by the addition of 2 μl of the appropriate diluted enzyme sample at 20 second intervals. For a control add 2 μl of enzyme dilution buffer in place of the enzyme to one sample.
6. Incubate for 15 minutes at 30 °C.
7. Stop reactions by the addition of 5 μl of the stop solution.
8. Boil for 5 minutes at 95 °C, then keep on ice.
9. Pre-run the gel for 30 minutes at 100 V, with circulating cold water to reduce heating.
10. Load 4 μl sample on 20% denaturing gel. Note: Wash the wells before loading.
11. Run the gel at 150 V with circulating cold water (~10 °C) to reduce heating until the stain front reaches 1 cm to 2 cm from the bottom of the gel (bromophenol blue and xylene cyanole FF run as an 8 base and 28 base oligonucleotides, respectively, on 20 % denaturing gels).
12. Carefully disassemble the gel and lay it on a piece of Whatman 3 mm paper. Cover the gel with a sheet of plastic wrap. Note: Do not dry the gel, it may crack.
13. Expose to X-ray film for 16 hours at –20 °C. It is recommended to put two layers of film on the gel in order to get at least one film properly exposed.

References

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