Endonuclease III (Nth)

**Source:** An *E. coli* strain which carries the cloned nth gene

**Applications:**
- Single cell gel electrophoresis (Comet assay) (3,4,5)
- Alkaline elution (6)
- Alkaline unwinding (7)

**Reagents Supplied with Enzyme:**
- 1X Endonuclease III (Nth) Reaction Buffer

**Reaction Conditions:**
- 1X Endonuclease III (Nth) Reaction Buffer: 20 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, pH 8.0 @ 25°C

**Unit Definition:**
- One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µl in 1 hour at 37°C.

**Endonuclease Activity:** Incubation of a 50 µl reaction containing 10 units of Endonuclease III in NEBuffer 1 with 1 µg αX174 RF I DNA for 4 hours at 37°C resulted in < 0.1% conversion to RFII as determined by agarose gel electrophoresis.

**Heat Inactivation:**
- 65°C for 20 minutes.

**References:**

Endonuclease III (Nth) protein from *E. coli* acts both as N-glycosylase and an AP-lyase. The N-glycosylase activity releases damaged pyrimidines from double-stranded DNA, generating a basic (AP site). The AP-lyase activity of the enzyme cleaves 3′ to the AP site leaving a 5′ phosphate and a 3′ -phospho-α, β-unsaturated aldehyde.

Some of the damaged bases recognized and removed by Endonuclease III include urea, 5, 6 dihydrouracil, uracil glycol, 6-hydroxy-5,6-dihydrothymine and methylthymine (1,2).

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**References:**

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**Quality Control Assays**

16-Hour Incubation:
- A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 25 units of Endonuclease III in NEBuffer 1 incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity:** Incubation of a 50 µl reaction containing 10 units of Endonuclease III in NEBuffer 1 with 1 µg of a mixture of single and double-stranded [*H] *E.coli* DNA (10³ cpmp/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

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**References:**