# Endonuclease III (Nth)

1-800-632-7799
info@neb.com
www.neb.com

M0268S 003150117011

# M0268S

**1,000 units** **10,000 U/ml** **Lot: 0031501**

**RECOMBINANT** **Store at –20°C** **Exp: 1/17**

**Description:** 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 Endouclease III include urea, 5, 6 dihydroxythymine, thymine glycol, 5-hydroxy-5 methylhydanton, uracil glycol, 6-hydroxy-5, 6-dihdrothimine and methyltartronylurea (1,2).

**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)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:**
10X Endonuclease III (Nth) Reaction Buffer.

**Reaction Conditions:** 1X Endonuclease III (Nth) Reaction Buffer. Incubate at 37°C.

**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.

*An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

**Assay Conditions**: 1X Endonuclease III (Nth) Reaction Buffer containing 10 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

**Recommended Dilution for the Comet Assay:** $1{:}10^4$ to $1{:}10^5$ (3,4,5). A detailed protocol can be found at www.neb.com

## 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 [$^3$H] *E.coli* DNA ($10^5$ cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

**Endonuclease Activity:** Incubation of a 50 µl reaction containing 10 units of Endonuclease III in NEBuffer 1 with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

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

**References:**

1. Dizdaroglu, M. Laval, J. and Boiteux, S. (1993) *Biochemistry* 32, 12105–12111.
2. Hatahet, Z. et al. (1994) *J. Biol. Chem.* 269, 18814–18820.
3. Singh, N. et al. (1988) *Experimental Cell Research* 175, 184–191.
4. Collins, A. et al. (1993) *Carcinogenesis* 14, 1733–1735.
5. Collins, A. et al. (1996) *Environmental Health Perspectives* 104, 465–469.
6. Pflaum, M. et al. (1998) *Free Rad. Res.* 29, 585–594.
7. Hartwig, A. et al. (1996) *Toxicology Letters* 88, 85–90.
8. Marks, K., New England Biolabs, Inc., unpublished observations.

CERTIFICATE OF ANALYSIS

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