Endonuclease III (Nth)

NEW ENGLAND BioLabs Inc.
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M0268S 003131115111

M0268S

1,000 units 10,000 U/ml Lot: 0031311

RECOMBINANT Store at –20°C Exp: 11/15

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.

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