While Endonuclease VIII is similar to Endonuclease III, Endonuclease VIII has β and δ lyase activity while Endonuclease III has β lyase activity.

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

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

**Reaction Conditions:** 1X Endonuclease VIII Reaction Buffer. Incubate at 37°C.

1X Endonuclease VIII Reaction Buffer:
- 10 mM Tris-HCl
- 75 mM NaCl
- 1 mM EDTA
- 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 VIII Reaction Buffer containing 10 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

**Recommended Dilution for Comet Assay:** 1:10^4 to 1:10^6 (3,4,5,8). A detailed protocol can be found at www.neb.com.

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Endonuclease VIII

**Description:** Endonuclease VIII from *E. coli* acts as both an N-glycosylase and an AP-lyase. The N-glycosylase activity releases damaged pyrimidines from double-stranded DNA, generating an apurinic (AP) site. The AP-lyase activity cleaves 3’ and 5’ to the AP site leaving a 5’ phosphate and a 3’ phosphate. Damaged bases recognized and removed by Endonuclease VIII include: uracil, 5, 6-dihydrothymine, thymine glycol, 5-hydroxy-5,6- dihydroxythymine and methyltartronylurea (1,2).

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

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

**Reaction Conditions:** 1X Endonuclease VIII Reaction Buffer. Incubate at 37°C.

1X Endonuclease VIII Reaction Buffer:
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**Recommended Dilution for Comet Assay:** 1:10^4 to 1:10^6 (3,4,5,8). A detailed protocol can be found at www.neb.com.

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Quality Control Assays

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 25 units of Endonuclease VIII 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 VIII with 1 µg of a mixture of single and double-stranded [3H] *E. coli* DNA (10^6 cpm/µg) for 4 hours at 37°C released < 0.4% of the total radioactivity

**Heat Inactivation:** 250 units of enzyme were inactivated by incubation at 75°C for 10 minutes.

**Usage Note:** Endonuclease VIII will remove deoxyribosyl 5’ phosphate dR5’P at a nicked site (9).
References:
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