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

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

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

**Reagents Supplied with Enzyme:**
- 10X Endonuclease VIII Reaction Buffer

**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.

**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.

**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 [³²P] *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 deoxyribos-5’ phosphate dR5’P at a nicked site (9).

(see other side)
References: