

## Endonuclease VIII



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



M0299S 009150316031

# M0299S



**1,000 units**    **10,000 U/ml**    **Lot: 0091503**  
**RECOMBINANT**    **Store at -20°C**    **Exp: 3/16**

**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 urea, 5, 6-dihydroxythymine, thymine glycol, 5-hydroxy-5-methylhydantoin, uracil glycol, 6-hydroxy-5, 6-dihydrothymine and methyltartronylurea (1,2).

While Endonuclease VIII is similar to Endonuclease III, Endonuclease VIII has  $\beta$  and  $\delta$  lyase activity while Endonuclease III has  $\beta$  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)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA and 50% glycerol.

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

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

## Endonuclease VIII



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



M0299S 009150316031

# M0299S



**1,000 units**    **10,000 U/ml**    **Lot: 0091503**  
**RECOMBINANT**    **Store at -20°C**    **Exp: 3/16**

**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 urea, 5, 6-dihydroxythymine, thymine glycol, 5-hydroxy-5-methylhydantoin, uracil glycol, 6-hydroxy-5, 6-dihydrothymine and methyltartronylurea (1,2).

While Endonuclease VIII is similar to Endonuclease III, Endonuclease VIII has  $\beta$  and  $\delta$  lyase activity while Endonuclease III has  $\beta$  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)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA and 50% glycerol.

**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  $\mu$ l 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  $\mu$ l.

### Recommended Dilution for Comet Assay:

1:10<sup>4</sup> to 1:10<sup>5</sup> (3,4,5,8). A detailed protocol can be found at [www.neb.com](http://www.neb.com).

### 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  $\mu$ l 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  $\mu$ l.

### Recommended Dilution for Comet Assay:

1:10<sup>4</sup> to 1:10<sup>5</sup> (3,4,5,8). A detailed protocol can be found at [www.neb.com](http://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  $\mu$ l reaction containing 1  $\mu$ g of  $\lambda$  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  $\mu$ l reaction containing 10 units of Endonuclease VIII with 1  $\mu$ g of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA (10<sup>5</sup> cpm/ $\mu$ g) 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 deoxyribose- 5' phosphate dR5'P at a nicked site (9).

(see other side)

CERTIFICATE OF ANALYSIS

### Quality Control Assays

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

**16-Hour Incubation:** A 50  $\mu$ l reaction containing 1  $\mu$ g of  $\lambda$  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  $\mu$ l reaction containing 10 units of Endonuclease VIII with 1  $\mu$ g of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA (10<sup>5</sup> cpm/ $\mu$ g) 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 deoxyribose- 5' phosphate dR5'P at a nicked site (9).

(see other side)

CERTIFICATE OF ANALYSIS

**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.
9. Marks, K., New England Biolabs, Inc., unpublished observations.

**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.
9. Marks, K., New England Biolabs, Inc., unpublished observations.