Endonuclease VIII

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0299S 009150116011

M0299S

RRI Yes

1,000 units	**10,000 U/ml**	**Lot: 0091501**
RECOMBINANT	**Store at –20°C**	**Exp: 1/16**

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-methylhydanton, uracil glycol, 6-hydroxy-5, 6-dihydrothymine and methyltartronylurea (1,2).

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 *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 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 $1{:}10^5$ (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 [^{3}H] *E. coli* DNA (10^5 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 deoxyribose- 5´ phosphate dR5´P at a nicked site (9).

(see other side)

CERTIFICATE OF ANALYSIS

Endonuclease VIII

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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.
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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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References:

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