hNEIL1

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

M0335S 002120512111

M0335S

500 units **5,000 U/ml** **Lot: 0021205**

RECOMBINANT **Store at –20°** **Exp: 11/12**

Description: Human NEIL1 acts as both an N-glycosylase and an AP-lyase. The N-glycosylase activity releases oxidized pyrimidines and purines 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 hNEIL1 include thymine glycol, 5,6-dihydrothymine, 5,6-dihydroxyuracil, 5-hydroxycytosine, 5-hydroxyuracil, 5-formyluracil, 8oxo-guanine opposite C, T or G, 4,6-diamino-5-formamidopyrimidine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (1,2).

Source: An *E. coli* strain that carries the cloned human NEIL1 gene.

Applications:

- Oxidative DNA damage studies
- Single cell gel electrophoresis (comet assay) (3,4,5)

Supplied in: 50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

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.

Molecular Weight: 43,685 Daltons

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Unit Assay Conditions: 1X Endonuclease VIII Reaction Buffer containing 5 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection. BSA is added to the enzyme for stabiity.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 25 units of hNEIL1 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 hNEIL1 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 < 0.4% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 25 units of hNEIL1 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: 20 minutes at 65°C.

(See other side)

CERTIFICATE OF ANALYSIS

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

1X Endonuclease VIII Reaction Buffer:
10 mM Tris-HCl
75 mM NaCl
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pH 8.0 @ 25°C

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50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

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Usage Note: Compared to the activity on AP-site, hNEIL1 has ≥ 50% activity on thymine glycol and ≥ 25% activity on dhU, dhT, 5hoU and 5hoC. hNEIL1 is active on 8oxoG opposite C,T and G, but is not active on an 8oxoG:A base pair. hNEIL1 is not active on 5-hydroxymethyluracil.

References:

1. Hazra, T.K. et al. (2002) *Proc. Natl. Acad. Sci.* 99, 3523–3528.
2. Bandaru, V. et al. (2002) *DNA Repair* 1, 517–529.
3. Singh, N., McCoy, M., Tice, R. and Schneider, L. (1988) *Experimental Cell Research* 175, 184–191.
4. Collins, A., Duthie, S. and Dobson, V. (1993) *Carcinogenesis* 14, 1733–1735.
5. Collins, A., Dusinska, M., Gedik, C. and Stetina, R. (1996) *Environmental Health Perspectives* 104, 465–469.
6. Katafuchi, A. et al. (2004) *J. Biol. Chem. 14,* 14464–14471.

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