

# hOGG1



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



M0241S 003120613061

## M0241S



**80 units**      **1,600 U/ml**      **Lot: 0031206**

**RECOMBINANT**    **Store at -20°C**    **Exp: 6/13**

**Description:** hOGG1 ( $\alpha$  isoform) is an 8-oxoguanine DNA glycosylase which acts both as a *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged purines from double stranded DNA, generating an apurinic (AP) site. The AP-lyase activity cleaves 3' to the AP site leaving a 5' phosphate and a 3'-phospho- $\alpha$ ,  $\beta$ -unsaturated aldehyde.

Some of the damaged bases recognized and removed by hOGG1 include 7, 8-dihydro-8-oxoguanine (8-oxoguanine) when base paired with

cytosine, 8-oxoadenine when base paired with cytosine, foramidopyrimidine (fapy)-guanine and methy-fapy-guanine (1,2).

**Source:** An *E. coli* strain that carries the cloned human *ogg1* gene (3).

#### Applications:

- Single cell gel electrophoresis (Comet assay) (4,5,6)
- Alkaline elution (7)
- Alkaline unwinding (8)

Supplied in: 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 50 mM NaCl, 200  $\mu$ g/ml BSA and 50% glycerol.

**Reagents supplied with Enzyme:**  
10X NEBuffer 2, 100X BSA.

**Reaction Conditions:** 1X NEBuffer 2, supplemented with 100  $\mu$ g/ml BSA. Incubate at 37°C.

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#### 1X NEBuffer 2:

50 mM NaCl  
10 mM Tris-HCl  
10 mM MgCl<sub>2</sub>  
1 mM DTT  
pH 7.9 @ 25°C

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**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 8-oxoguanine base paired with a cytosine in 10  $\mu$ l of 1X NEBuffer 2 containing 10 pmol of substrate, supplemented with 100  $\mu$ g/ml BSA in 1 hour at 37°C.

**Unit Assay Conditions:** 1X NEBuffer 2 containing 10 pmol of fluorescently labeled oligonucleotide duplex, supplemented with 100  $\mu$ g/ml BSA.

**Recommended dilution for the Comet Assay:** 1:10<sup>2</sup> to 1:10<sup>3</sup> (4,5,6,9). A detailed protocol can be found at [www.neb.com](http://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. BSA is added to the enzyme for stability.

**16-Hour Incubation:** A 50  $\mu$ l reaction containing 1  $\mu$ g of  $\lambda$  DNA (HindIII digest) and 16 units of hOGG1 incubated for 16 hours at 37°C resulted in DNA patterns free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity:** Incubation of a 50  $\mu$ l reaction containing 8 units of hOGG1 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 < 0.1% of the total radioactivity.

**Endonuclease Activity:** Incubation of 8 units of enzyme with 1  $\mu$ g  $\phi$ X174 RF I DNA for 4 hours at 37°C in 50  $\mu$ l reaction buffer resulted in < 5% conversion to RF II.

(See other side)

CERTIFICATE OF ANALYSIS

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**Heat Inactivation:** 160 units of enzyme were inactivated by incubation at 65°C for 15 minutes.

**References:**

1. Bjoras, M. et al. (1997). Opposite base-dependent reactions of a human base excision repair enzyme on DNA containing 7, 8-dihydro-8-oxoguanine and abasic sites. *EMBO J.* 16, 6314–6322.
2. Boiteux, S. and Radicella, J. (1999). Base excision repair of 8-hydroxyguanine protects DNA from endogenous oxidative stress. *Biochimie* 81, 59–67.
3. Radicella, J., Dherin, C. Desmze, C., Fox, M. and Boiteux, S. (1997). Cloning and characterization of *hOGG1*, a human homolog of the *OGG1* gene of *Saccharomyces cerevisiae*. *PNAS USA* 94, 8018–8015.
4. Singh, N., McCoy, M., Tice, R. and Schneider, L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research* 175, 184–191.

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5. Collins, A., Duthie, S. and Dobson, V. (1993). Direct enzymatic detection of endogenous oxidative base damage in human lymphocyte DNA. *Carcinogenesis* 14, 1733–1735.
6. Collins, A., Dusinska, M., Gedik, C. and Stetina, R. (1996). Oxidative damage to DNA: do we have a reliable biomarker? *Environmental Health Perspectives* 104, 465–469.
7. Pflaum, M., Will, O., Mahler, H-C. and Epe, B. (1998). DNA oxidation products determined with repair endonucleases in mammalian cells: types, basal levels and influence of cell proliferation. *Free Rad. Res.* 29, 585–594
8. Hartwig, A., Dally, H. and Schlepegrell, R. (1996). Sensitive analysis of oxidative DNA damage in mammalian cells: use of the bacterial Fpg protein in combination with alkaline unwinding. *Toxicology Letters* 88, 85–90.
9. Guthrie, E. New England Biolabs, Inc. unpublished observations

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