hOGG1

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

M0241S 003151016101

M0241S

RR BSA Yes

80 units **1,600 U/ml** **Lot: 0031510**

RECOMBINANT **Store at –20°C** **Exp: 10/16**

Description: hOGG1 (α 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-α, β-unsaturated aldehyde.

Some of the damaged bases recognized and removed by hOGG1 include 7, 8-dihydro-8-oxo-guanine (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 µg/ml BSA and 50% glycerol.

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

Reaction Conditions: 1X NEBuffer 2, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
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 µl of 1X NEBuffer 2 containing 10 pmol of substrate, supplemented with 100 µg/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 µg/ml BSA.

Recommended dilution for the Comet Assay:
$1{:}10^2$ to $1{:}10^3$ (4,5,6,9). 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. BSA is added to the enzyme for stability.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ 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 ul reaction containing 8 units of hOGG1 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 < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of 8 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl 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 *hOGG*1, a human homolog of the *OGG*1 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.
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. Gutherie, E. New England Biolabs, Inc. unpublished observations

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