gene (3). 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-oxoguanine (8-oxoguanine) when base paired with cytosine, 8-oxoadenine when base paired with cytosine, formamidopyrimidine (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)

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 MgCl₂
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 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: 1:10^3 to 1:10^4 (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 µl reaction containing 8 units of hOGG1 with 1 µg of a mixture of single and double-stranded [3H] E. coli DNA (10⁶ 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)
Heat Inactivation: 160 units of enzyme were inactivated by incubation at 65°C for 15 minutes.

References: