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)

**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 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:** 1:10² to 1:10³ (4,5,6,9). A detailed protocol can be found at 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 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 (10⁵ 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.

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(See other side)
Heat Inactivation: 160 units of enzyme were inactivated by incubation at 65°C for 15 minutes.

References:

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

References: