**Product Description:** Fpg (also known as Formamidopyrimidine DNA glycosylase, Mut M, FAPY DNA Glycosylase, and 8-oxoguanine DNA glycosylase) participates in the base-excision (BER) pathway of DNA repair enzymes and acts both as a N-glycosylase and an AP-lyase. The N-glycosylase activity releases damaged purines from double stranded DNA, generating an apurinic/apyrimidinic (AP site). The AP-lyase activity cleaves both the 3' and 5' phosphodiester bonds at the AP site, producing a 1 base gap in the DNA and 3' and 5' phosphate termini. Bases recognized and removed by Fpg include 7, 8-dihydro-8-oxoguanine (8-oxoguanine), 8-oxoadenine, fapy-guanine, methy-fapy-guanine, fapy-adenine, aflatoxin B1-fapy-guanine, 5-hydroxy-cytosine and 5-hydroxy-uracil (1,2).

<table>
<thead>
<tr>
<th><strong>Source of Protein:</strong></th>
<th>A recombinant E. coli strain carrying the cloned fpg gene.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit Definition:</strong></td>
<td>1 unit is defined as the amount of enzyme required to cleave 1 pmol of a 34mer oligo-nucleotide duplex containing an 8-oxoguanine base paired with a cysteine in 1 hour at 37°C in a total reaction volume of 10µl in reaction buffer.</td>
</tr>
<tr>
<td><strong>Molecular weight:</strong></td>
<td>30,290 Daltons</td>
</tr>
<tr>
<td><strong>Quality Control Analysis:</strong></td>
<td></td>
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<tr>
<td><strong>Unit Activity</strong> is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and added to 10 µL reactions containing 1X Yellow Buffer (B0130) and a FAM-labeled duplex oligonucleotide, containing a single 8-oxoguanine site. Reactions were incubated 1 hour at 37°C, plunged on ice, denatured with N-N-dimethylformamide and analyzed on a 15% TBE-Urea acrylamide gel.</td>
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</tr>
<tr>
<td><strong>Protein Concentration (OD&lt;sub&gt;280&lt;/sub&gt;)</strong> is determined by OD&lt;sub&gt;280&lt;/sub&gt; absorbance.</td>
<td></td>
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<tr>
<td><strong>Physical Purity</strong> is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.</td>
<td></td>
</tr>
<tr>
<td><strong>Single-Stranded Exonuclease</strong> is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.</td>
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</tr>
<tr>
<td><strong>Double-Stranded Exonuclease</strong> is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.</td>
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</tbody>
</table>
**E.coli 16S rDNA Contamination** is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

**Supplied in:** 20mM Tris-HCL, 50 mM KCl, 1mM DTT, 0.1mM EDTA, 50% glycerol pH 8.0 @ 25°C.

**Supplied with:**

10X Yellow Buffer (B0130): 100mM Bis-Tris Propane, 100mM MgCl₂, 10mM DTT pH 7.0 @ 25°C.

**Notes:** Fpg is a DNA repair enzyme which cleaves the phosphodiester bond at abasic sites, a common form of naturally occurring DNA damage. Following thorough characterization of the Fpg enzyme in our nuclease quality control tests, both during and after purification, we have concluded the inherent presence of abasic sites in DNA substrates contributes to false positives in tests for exogenous endo and exonuclease contaminants.

**Usage Information:**

**Protocol for Comet Assay:**

After lysis of cells/nuclei embedded in low melting temperature agarose:

1. Add 100 µl of 1X reaction buffer per slide and apply cover slip.
2. Equilibrate 5 minutes.
3. Remove cover slip, then tap slide on its side to remove excess reaction buffer.
4. Dilute Fpg 82X in 1X Yellow buffer.
5. Add 100 µl dilute Fpg solution per slide and replace cover slip.
6. Incubate slide at 37°C for 30 minutes.
7. Proceed with further enzymatic manipulation or continue with alkali unwinding.

**References:**


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**Limitations of Use**

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.