**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).

**Source of Protein**

A recombinant *E. coli* strain carrying the cloned *fpg* gene.

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### Product Information

<table>
<thead>
<tr>
<th><strong>E. coli Fpg</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Part Number</strong></td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
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<tr>
<td><strong>Unit Size</strong></td>
</tr>
</tbody>
</table>

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### Product Specification

<table>
<thead>
<tr>
<th><strong>Storage Temperature</strong></th>
<th>-25°C to -15°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purity (SDS-PAGE)</strong></td>
<td>&gt;99%</td>
</tr>
<tr>
<td><strong>Specific Activity</strong></td>
<td>20,513 U/mg</td>
</tr>
<tr>
<td><strong>SS Exonuclease</strong></td>
<td>80 U &lt;10% released</td>
</tr>
<tr>
<td><strong>DS Exonuclease</strong></td>
<td>80 U &lt;1.0% released</td>
</tr>
<tr>
<td><strong>E. coli DNA Contamination</strong></td>
<td>80 U &lt;10 copies</td>
</tr>
</tbody>
</table>

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### Unit Definition

One 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.

### Quality Control Analysis:

**Unit Characterization Assay**

Specific activity was 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.

### Protein Concentration (OD\(_{280}\)) Measurement

A 2.0 µL sample of enzyme was analyzed at OD\(_{280}\) using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209) and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 38,690 and molecular weight of 30,290 Daltons.

### SDS-Page (Physical Purity Assessment)

2.0 µL of enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µL of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.
Contamination Tests:

**Single-Stranded Exonuclease Activity**
A 50 µL reaction containing 10,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 10% release of TCA-soluble counts.

**Double-Stranded Exonuclease Activity**
A 50 µL reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 1.0% release of TCA-soluble counts.

**E.coli 16S rDNA Contamination Test**
Replicate 5 µL samples of enzyme solution were 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. The acceptance criterion for the test is the threshold cycle count ($C_t$) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control $C_t$ values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

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 instructions:
**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.
Proceed with further enzymatic manipulation or continue with alkali unwinding.

References: