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.

Supplied in
20 mM Tris-HCl
50 mM NaCl
1.0 mM dithiothreitol
0.5 mM EDTA
50% glycerol
pH 8.0 @ 25°C

Supplied With
B907 10X Fpg Reaction Buffer

10X Fpg Reaction Buffer (B907)
100 mM Tris-HCl
100 mM KCl
20 mM EDTA
pH 7.9 @ 25°C

Unit Definition
One unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of [32P] (ATP donor) in 30 minutes at 37°C in 1X T4 Polynucleotide Kinase Reaction Buffer.

Quality Control Analysis:
Unit Characterization Assay
Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme batch were made in Fpg glycerol storage solution and added to 10 µL reactions containing 1X Fpg Reaction Buffer and 2.0 µM of a FAM-labeled, 34-base, duplex oligonucleotide, containing a single Uracil. [Note: substrate pre-treated for 2 minutes with 1 unit of UDG to create an abasic site] Reactions were incubated 15 minutes at 37°C, plunged on ice, denatured with N-N-dimethylformamide and analyzed by running and exposing to short-wave UV 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. Acceptance for this assay is +/- 5% of reference sample.
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.

Nuclease Contamination Tests:
Single-Stranded Endonuclease Activity
A 50 µL reaction containing 15,000 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 0.1% release of TCA-soluble counts.

Double-Stranded Endonuclease Activity
A 50 µL reaction containing 15,000 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 0.2% 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 (Ct) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control Ct values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

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 reaction 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.

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.

References: