



Product Information	
<i>E. coli</i> Fpg	
Part Number:	Y907L
Lot Number:	

Certificate of Analysis

2010-Y907-Rev.D

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/aprimidinic (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

B013 10X Yellow Buffer

10X Yellow Buffer (B013)

100 mM BisTris-Propane-HCl
100 mM MgCl₂
10 mM dithiothreitol
pH 7.0 @ 25°C

(additional information on reverse side)

Product Specification*	
Unit Size:	4,000 Units
Unit Concentration	8,000 U/mL
Protein Concentration	0.39 mg/ml
Purity (SDS-PAGE)	>99%
Specific Activity (est.)	20,513 U/mg
SS Exonuclease	80 U <5.0% released
DS Exonuclease	80 U <0.5% released
<i>E. coli</i> 16S rDNA Contamination	80 U <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below

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 in 1 hour at 37°C in 1X Yellow 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 ([Fpg]_f = 0.4 – 0.05 μg/μL) and added to 10 μL reactions containing 1X Yellow Buffer and 1.0 μM of a FAM-labeled, 34-base, 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 (OD₂₈₀) Measurement

A 2.0 μL sample of enzyme was analyzed at OD₂₈₀ 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 11,000 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 5.0% release of TCA-soluble counts.

Double-Stranded Endonuclease 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 0.5% 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.

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.

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:

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3. Boiteux, S., O'Connor, T. and Laval, J. (1987) Formamidopyrimidine-DNA glycosylase of *Escherichia coli*: cloning and sequencing of the fpg structural gene and overproduction of the protein. *EMBO J.*, 5, 3177-3183.
4. Singh, N., McCoy, M., Tice, R. and Schneider, L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.*, 175, 184-191.
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8. Hartwig, A., Dally, H. and Schlegel, R. (1996) Sensitive analysis of oxidative DNA damage in mammalian cells: use of the bacterial Fpg protein in combination with alkaline unwinding. *Toxicol. Lett.*, 88, 85-90.
9. Czene, S. and Harms-Ringdahl, M. (1995) Detection of single strand breaks and formamidopyrimidine-DNA glycosylase-sensitive sites in DNA of cultured human fibroblasts. *Mutat. Res.*, 336, 235-242.



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. MSDS sheets relevant to this product are available upon request.