

TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

E. coli Formamidopyrimidine-DNA Glycosylase (Fpg)

Catalog #: 4040-100-EB

Contents:	4040-100-01	Fpg	Size:	500 Units
	3900-500-10	10X REC™ Buffer 10		1 ml

Description: Fpg releases damaged bases preferentially from duplex DNA. It has an associated class I AP lyase activity, leaving both 3' and 5' phosphoryl groups. This results from a β , δ elimination reaction at the AP sites, producing a single nucleotide gap in the DNA. The enzyme consists of 269 amino acids with a molecular weight of 30.2 kDa.

Source: Purified from *E. coli* containing a recombinant plasmid harboring the *E. coli* fpg gene.

Unit Definition: One Unit is the amount of enzyme required to cleave 1 pmole of a ³²P-labeled oligonucleotide probe containing 8-oxoguanine, within an oligonucleotide duplex in one hour at 37 °C.

Specificity: Fpg catalyzes the excision of the following forms of DNA damage:

1. Open ring forms of 7-methylguanine, including 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine and 4,6-diamino-5-amidopyrimidine, a lethal lesion.
2. 8-oxoguanine, a highly mutagenic lesion and probably the most important biological substrate of Fpg.
3. 5-hydroxycytosine
4. 5-hydroxyuracil
5. Aflatoxin-bound imidazole-ring-opened guanine
6. Imidazole ring opened N-2-aminofluorene-C8-guanine

Assay Conditions & Analysis: 1X REC Buffer 10 (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 10 mM EDTA, and 0.1 mg/ml BSA), 4 pmoles of 8-oxo-dG Oligonucleotide (Cat# 3850-100-01) labeled with ³²P, 4 pmoles of Oligo Complement A (Cat# 3849-100-01), and serial dilutions of enzyme in a reaction volume of 20 μ l are incubated for 1 hour at 37°C. For analysis, 10 μ l of 3X REC Alkali Loading Buffer (Cat# 4017-500: 300 mM NaOH, 97% formamide, and 0.2% bromophenol blue) are added, the samples are heated to 95°C for 10 min then fast cooled to 4°C, and the cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. The bands were cut out and radioactivity counted to quantify the cleavage products.

Storage Buffer: 20 mM Tris-Cl (pH 7.8), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol.

Storage Conditions: Store at -20°C in a manual defrost freezer. For long term storage, freeze in working aliquots at -80°C. Avoid repeated freeze-thawings. May be diluted in 10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 50% glycerol and store at -20°C for up to 1 week. Otherwise, dilute enzyme in 1X REC Buffer 10 and use immediately. It is stable for up to 8 hours at 37°C without any loss in activity.

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E6/24/08v1

TREVIGEN®

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References:

1. Tchou, J., V. Bodepudi, S. Shibutani, I. Antoshechkin, J. Miller, A.P. Grollman, and F. Johnson. 1994. Substrate specificity of Fpg protein: recognition and cleavage of oxidatively damaged DNA. *J Biol Chem* **269**: 15318-15324.
2. Friedberg, E.C., G.C. Walker, and W. Siede. 1995. DNA Repair and Mutagenesis. American Society of Microbiology, Washington, D.C.: ASM Press.
3. Boiteux, S., T.R. O'Connor, and J. Laval. 1987. Formamidopyrimidine-DNA glycosylase of *Escherichia coli*: cloning and sequencing of the Fpg structural gene and overproduction of the protein. *EMBO J* **6**: 3177-3183.

Lot number:
Concentration:
Units:
Specific Activity:

E. coli
Formamidopyrimidine
DNA Glycosylase (Fpg)
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