

# TREVIGEN® Product Data

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## *E. coli* Formamidopyrimidine-DNA Glycosylase (Fpg)

**Catalog #:** 4040-500-EB

<b>Contents:</b>	4040-100-01	Fpg	<b>Size:</b>	5 x 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  $\beta$ ,  $\delta$  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 cleaves 1 pmole of a 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 labeled 8-oxo-dG oligonucleotide annealed to the compliment oligonucleotide, and serial dilutions of enzyme in a reaction volume of 20  $\mu$ l are incubated for 1 hour at 37°C. For analysis, 20  $\mu$ l of 2X Loading Buffer (20 mM EDTA, 95% formamide, and 0.13% 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, and percent cleavage quantified.

**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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## 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.

**DNA Repair Enzymes:**

Catalog #	Description	Size
4150-010-EB	<i>Sulfolobus solfataricus</i> DNA Polymerase IV (Dpo4)	10 µg
4045-01K-EB	<i>E. coli</i> Endonuclease III	1000 U
4050-100-EB	<i>E. coli</i> Endonuclease IV	100 U
4065-100-EB	Chorella Virus Pyrimidine Dimer Glycosylase	1000 U
4130-100-EB	Human 8-oxo-G DNA Glycosylase (hOGG1)	100 U
4100-100-EB	<i>S. pombe</i> UVDE	100 µl
4025-100-EB	<i>E. coli</i> Uracil-N-Glycosylase	100 U
4000-500-EB	<i>E. coli</i> MutY DNA Glycosylase	500 U
4110-01K-EB	Human AP Endonuclease	1000 U
4020-01K-EB	Human β Polymerase	1000 U
4120-100-EB	Human FEN-1	100 U
4125-100-EB	<i>E. coli</i> Mismatch Uracil DNA Glycosylase (Mug)	100 U
4090-100-EB	Mouse 3-Methyladenine DNA Glycosylase (Aag)	100 U
4070-500-EB	Thermostable thymine mismatch DNA Glycosylase	500 U
4055-100-EB	T4 Endonuclease V	100,000 U

***E. coli***  
**Formamidopyrimidine**  
**DNA Glycosylase (Fpg)**  
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