E. coli Formamidopyrimidine-DNA Glycosylase (Fpg)

Catalog #: 4040-100-EB
4040-500-EB

Size: 500 Units
2500 Units

Contents: Fpg
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 ^{32}P-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-OL) labeled with ^{32}P, 4 pmoles of Oligo Complement A (Cat.# 3850-100-OL), and serial dilutions of enzyme in a reaction volume of 20 µL are incubated for 1 hour at 37° C. For analysis, 10 µL of 3X Alkali Loading Buffer (300 mM NaOH, 97% formamide, and 0.2% bromophenol blue) are added, the samples are heated to 95° C for 10 minutes then fast cooled to 2 - 8° C, and the cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. The bands were cut out and the radioactivity is counted to quantify the cleavage products.

Storage Buffer: 10 mM HEPES-KOH (pH 7.4), 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, aliquot and store at -80° C. Avoid repeated freeze-thaw cycles. 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 stored at -20° C for up to 1 week. Otherwise, dilute enzyme in 1X REC Buffer 10 and use immediately. Fpg is stable for up to 8 hours at 37° C without any loss in activity.

References: see reverse.

R&D Systems, Inc.
614 McKinley Place N.E., Minneapolis, MN 55413 USA
Tel: 1-800-343-7475 • 612-379-2956 • Fax: 612-379-6580
e-mail: info@RnDSystems.com • www.RnDSystems.com

References:

1. Tchou, J., *et al.* (1994) *Substrate specificity of Fpg protein: recognition and cleavage of oxidatively damaged DNA*. J. Biol. Chem. **269**:15318.
2. Friedberg, E.C., *et al.* (1995) *DNA Repair and Mutagenesis*. American Society of Microbiology, Washington, D.C.: ASM Press.
3. Boiteux, S., *et al.* (1987) *Formamidopyrimidine-DNA glycosylase of Escherichia coli: cloning and sequencing of the Fpg structural gene and overproduction of the protein.* EMBO J. **6**:3177.

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