

Fpg



1-800-632-7799
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www.neb.com



M0240S 004130314031

M0240S



500 units **8,000 U/ml** **Lot: 0041303**
RECOMBINANT **Store at -20°C** **Exp: 3/14**

Description: Fpg (formamidopyrimidine [fapy]-DNA glycosylase) (also known as 8-oxoguanine DNA glycosylase) acts both as a *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged purines from double-stranded DNA, generating an apurinic (AP site). The AP-lyase activity cleaves both 3' and 5' to the AP site thereby removing the AP site and leaving a 1 base gap with a 5' and 3' phosphate.

Some of the damaged 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: An *E. coli* strain that carries the cloned *fpg* gene (3)

Applications:

- Single cell gel electrophoresis (Comet assay) (4,5,6)
- Alkaline elution (7)
- Alkaline unwinding (8)
- Modified nick translation (9)

Supplied in: 20 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 50 mM NaCl, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

10X NEBuffer 1, 100X BSA.

Reaction Conditions: 1X NEBuffer 1, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 1:

10 mM Bis Tris Propane-HCl
10 mM MgCl₂
1 mM DTT
pH 7.0 @ 25°C

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34-mer oligonucleotide duplex containing a single 8-oxoguanine base paired with a cytosine in a total reaction volume of 10 µl in 1 hour at 37°C.

Unit Assay Conditions: 1X NEBuffer 1 containing 10 pmol of fluorescently labeled oligonucleotide duplex, supplemented with 100 µg/ml BSA in a total reaction volume of 10 µl.

Recommended Dilution for the Comet Assay:

1:10³ to 1:10⁴ (4,5,6,10). A detailed protocol can be found at www.neb.com.

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 40 units of Fpg incubated for 16 hours at 37°C resulted in DNA patterns free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 40 units of Fpg with 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA (10⁵ cpm/µg) for 4 hours at 37°C released < 1.0% of the total radioactivity.

Heat Inactivation: 160 units of enzyme were inactivated by incubation at 60°C for 10 minutes.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection. BSA is added to the enzyme for stability.

Usage Note: Fpg will remove deoxyribose- 5' phosphate dR5'P at a nicked site (11).

(see other side)

CERTIFICATE OF ANALYSIS

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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. *Experimental Cell Research* 175, 184–191.
5. Collins, A., Duthie, S. and Dobson, V. (1993). Direct enzymatic detection of endogenous oxidative base damage in human lymphocyte DNA. *Carcinogenesis* 14, 1733–1735.
6. Collins, A., Dusinska, M., Gedik, C. and Stetina, R. (1996). Oxidative damage to DNA: do we have a reliable biomarker? *Environmental Health Perspectives* 104, 465–469.
7. Pflaum, M., Will, O., Mahler, H-C. and Epe, B. (1998). DNA oxidation products determined with repair endonucleases in mammalian cells: types, basal levels and influence of cell proliferation. *Free Rad. Res.* 29, 585–594
8. Hartwig, A., Dally, H. and Schlepegrell, R. (1996). Sensitive analysis of oxidative DNA damage in mammalian cells: use of the bacterial Fpg protein in combination with alkaline unwinding. *Toxicology Letters* 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. *Mutation Research* 336, 235–242.
10. Guthrie, E., New England Biolabs, Inc., unpublished observations.
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