Fpg

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

M0240S 0041210131O1

M0240S

RR BSA Yes

500 units	**8,000 U/ml**	**Lot: 0041210**
RECOMBINANT	**Store at –20°C**	**Exp: 10/13**

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 B_1-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 $MgCl_2$
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:
1:10^3 to 1:10^4 (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 ul reaction containing 40 units of Fpg with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^5 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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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.
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Page 2 (M0240)