MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.0

Product name: Fpg, *E. coli*

Cat #: FPG-100, FPG-200, FPG-OEM, B-FB10

Description:

Fpg (also known as Formamidopyrimidine DNA glycosylase, MutM, FAPY DNA Glycosylase, and 8-oxoguanine DNA glycosylase) participates in the base-excision (BER) pathway of DNA repair enzymes and acts both as a N-glycosylase and an AP-lyase. The N-glycosylase activity releases damaged purines from double stranded DNA, generating an apurinic/apyrimidinic (AP site). The AP-lyase activity cleaves both the 3′ and 5′ phosphodiester bonds at the AP site, producing a 1 base gap in the DNA and 3′ and 5′ phosphate termini. 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.

Protocol:

Enzyme treatment Fpg:
Prepare 1X enzyme reaction buffer.
Reserve 1 ml for dilutions of the enzyme.
Wash slides in a staining jar with enzyme buffer 3 times, 5 minutes for each wash.
Remove slides from the last wash.
Dab off the excess liquid with tissue.
Place 50 µl of the enzyme solution or buffer alone onto gel surface and cover with a 22 x 22 mm cover slip.
Put slides in a moist box (tupperware container with damp paper towels) and incubate at 37°C for 30 minutes.
While the amount of the enzyme required should be determined for each cell type and experiment we suggest a first try with a 1:103 to 104 dilution of Fpg.