**Product Information**

**Fpg Protein**  
(\textit{Formamidopyrimidine DNA glycosylase, Fapy DNA glycosylase, MutM})  
\textit{E. coli}, Recombinant

**Product Number** F 3174  
**Storage Temperature** –20°C

**Product Description**  
This product is an \textit{E. coli} recombinant protein expressed in \textit{E. coli}.

Fpg Protein (EC 3.2.2.23) is a key enzyme in the base excision repair pathway (BER) and a member of the DNA repair process\textsuperscript{1}. It catalyses the excision of a broad spectrum of modified purines such as formamidopyrimidine (Fapy) and of 8-oxo-guanine (8-oxo-G), which is a major spontaneous oxidative DNA product.

Fpg Protein possess both DNA glycosylase activity, which removes the mutated base and (apurinic/apyrimidinic) AP-lyase activity that releases the ribose, leaving both 5’-phosphoryl and 3’-phosphoryl groups in the DNA\textsuperscript{2}. Several analytical methods based on Fpg Protein activity \textit{in vitro} were developed for detection and quantitation of oxidative damage to DNA mainly for FapyA, FapyG and 8-oxo-G\textsuperscript{3,4}.

The \textit{fpg} gene was cloned by Boiteux, et al.\textsuperscript{5} The protein predicted from the nucleotide sequence composed of 269 amino acids with a molecular weight of 30.2 kDa. Fpg Protein possesses a zinc finger motif at its C-terminal (one zinc atom per molecule). This region is responsible for the DNA binding and AP-lyase activity of Fpg enzyme.\textsuperscript{6} In addition, its N-terminal proline-1 was found to act as a nucleophile to produce a Schiff base intermediate, which is essential for the enzyme action.\textsuperscript{2}

**Reagents**

Vial content: A solution in 50% glycerol containing 50 mM K-HEPES, pH 7.5, 1 mM DTT, 1 mM EDTA, 200 mM NaCl

Purity: > 90% by SDS-PAGE.
Specific activity: >20,000 units per mg protein.

Unit definition: The amount of protein that cleaves 50% of 0.5 pmol double strand DNA oligomer substrate (8-oxo guanine mutated) in 10 min at 25°C.

**Storage/Stability**  
Store at –20°C.

**Preparation Instruction**  

1. **32P labeled Fpg Protein substrate ds-oligonucleotide**. Prepare the substrate according the technical bulletin for the FPG Protein Substrate Set, (Product Code F9550).
2. **10X reaction buffer**: 500 mM Tris pH 7.6, 20 mM EDTA, 500 mM KCl.
3. **Enzyme dilution buffer**: 50 mM K-HEPES, pH 7.6, 1 mM EDTA, 1 mM DTT, 10% v/v glycerol, 200 mM NaCl.
4. **Stop solution**: 90% (v/v) formamide, 0.1% (w/v) Bromophenol blue, 0.1% (w/v) xylene cyanole, 20 mM EDTA.
5. 20% denaturing gel.
6. **Running buffer – TBE**: 89 mM Tris base, 2 mM EDTA, 89 mM Boric acid, pH 8.0.
7. X-Ray film and developing machine

**Procedure**

Assay principle
The Fpg Protein activity assay is based on its glycosylase activity that recognizes and removes the mutated base (8-oxo-G) which is followed by its lyase activity that cleaves the AP (apurinic) strand of the double strand DNA. The substrate used for assaying the activity of Fpg protein is a radiolabeled double stranded-23 oligonucleotide containing 8-oxo-dG at the 11th base of the labeled strand (Product Code F9550, Fpg protein substrate set).
In the reaction, Fpg first removes the 8-oxo-G base and then cleaves the mutated strand at the apurinic site. Denaturation of the double strands and separation on denatured polyacrylamide gel produces two labeled bands: a 23 bp oligonucleotide band (residual uncleaved substrate) and a 10 bp oligonucleotide band (the cleavage product).

1. Prepare 20% denaturing gel containing 7 M urea, assemble the electrophoresis apparatus and add running buffer.
2. Prepare reaction mix for 10 runs:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount per 10 runs</th>
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<tbody>
<tr>
<td>10X Reaction Buffer</td>
<td>10 µl</td>
</tr>
<tr>
<td>32P labeled substrate</td>
<td>2 µl (~5 pmol)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>68 µl</td>
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</tbody>
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3. Dilute Fpg enzyme to 1,5,10, and 20 µg/ml with enzyme dilution buffer.
4. Dispense 8 µl of reaction mix to each tube.
5. Start the reaction by the addition of 2 µl diluted enzyme samples using 20-second intervals. For a control add 2 µl of enzyme dilution buffer to one sample.
6. Incubate for 10 min at 25°C.
7. Stop reactions by the addition of 5 µl stop solution.
8. Load 4 µl sample on 20% denaturing gel. Note: wash the wells before loading.
9. Run the gel at 150V with circulating cold water (~10°C) to reduce heating until the stain front reaches 1-2 cm from the bottom of the gel (bromophenol blue and xylene cyanole run as 8 and 28 base oligonucleotide on 20% denaturing gels).
10. Carefully disassemble the gel and lay it on a piece of Whatman 3 mm paper. Cover the gel with a sheet of plastic wrap. **Note: do not dry the gel, it may crack.**
11. Expose to X-ray film for 16 hr at −20°C. It is recommended to put two layers of film on the gel in order to get at least one gel properly exposed.

**References**


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