Methylpurine DNA N-Glycosylase
Human, Recombinant

Product Number M 7940
Storage Temperature −70 °C

Product Description
Human methylpurine DNA N-glycosylase (MPG, AAG) is a 32 kDa protein comprised of 298 amino acids. MPG is expressed in E. coli as a MBP fusion protein resulting in an apparent MW of approximately 70 kDa. MPG is a base excision repair (BER) protein that removes the mutated N-methyl purine nucleotide from alkylated DNA, creating an apurinic/apyrimidinic (AP) site. MPG recognizes and excises 3-methyladenine, 7-methylguanine, 3-methylguanine, N-6-ethanol-adenine and hypoxanthine.

Reagent
This product is supplied as a solution in 50% Glycerol (w/v) containing 20 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, 250 mM NaCl, 0.25% CHAPS, and 1/100 (v/v) Protease Inhibitor Cocktail (Product No. P 8340).

Purity: minimum 80% (SDS-PAGE)
Specific Activity: minimum 5,000 units per mg protein (Calculated according to protein MW of 70 kDa).

Unit Definition: The amount of protein that cleaves 50% of 0.5 pmole of double-stranded DNA oligomer substrate containing a hypoxanthine site lesion in 10 minutes at 37 °C.

Precautions and Disclaimer
This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and storage at −70 °C is recommended.

Procedure
The MPG activity assay is based on its glycosylase activity that recognizes and removes the mutated base hypoxanthine, producing an apurinic/apyrimidinic (AP) site.

For the assay of this enzyme, a 24-base oligonucleotide containing hypoxanthine at the 14 base position is 32P-labeled by polynucleotide kinase (PNK) and annealed to a complementary strand having thymidine opposite to the hypoxanthine. During the reaction, MPG removes the hypoxanthine from the labeled first strand and the abasic site is then nicked by 1,4-diaminobutane (putrescine) that is added to the reaction mix. Denaturation of the double-stranded oligonucleotides and separation on a denaturing (7 M urea) polyacrylamide gel results in the appearance of a 14 base labeled band in addition to the original 24 base band. The substrate used in this assay is:

First strand: 5' TTGGTTGGTGGTHGGTGGTGGGG 3'
where H is hypoxanthine

Complementary strand: 5' CCCCAACACCTACCACCCAACCAA 3'

Reagents and Equipment Needed but not supplied
- T4 polynucleotide kinase (PNK) (Product No. P 4390)
- T4 polynucleotide kinase (PNK) buffer
- 32P-ATP 10 mCi/ml
- 10X Reaction Buffer: Prepare 10 ml of 500 mM HEPES, pH 7.5, containing 100 mM MgCl2, 500 mM KCl, 0.5% Triton X-100, and 10 μg/ml bovine serum albumin
- Enzyme Dilution Buffer: 20 mM Tris-HCl, pH 7.5, containing 1 mM EDTA, 1 mM DTT, 10% (v/v) glycerol, and 200 mM NaCl
- Stop Solution: 90% formamide, 0.1% (w/v) bromophenol blue, 0.1% (w/v) xylene cyanole FF, and 20 mM EDTA
- 1,4-diaminobutane, 0.5 M, pH 8 (Product No. P 7630)
- Desalting column (G-25 microspin column) for 50 μl sample
- 20% denaturing (7 M urea) acrylamide gel and electrophoresis apparatus
- Tris-Borate-EDTA (TBE) gel running buffer (Product No. T 9525)
- Whatman 3 mm paper
- X-ray film and developing machine
A. Preparation of Double-Stranded Oligonucleotide Substrate
1. Prepare the following mix:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x PNK Buffer</td>
<td>3 µl</td>
</tr>
<tr>
<td>First Strand Oligonucleotide</td>
<td>10 µl (100 pmole)</td>
</tr>
<tr>
<td>γ²³P-ATP 10 mCi/ml</td>
<td>3 µl (30 µCi)</td>
</tr>
<tr>
<td>T4 PNK</td>
<td>1 µl</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>13 µl (30 µl total)</td>
</tr>
</tbody>
</table>

2. Incubate for 60 minutes at 37 °C.
3. Inactivate for 10 minutes at 70 °C.
4. Remove unincorporated ATP using G-25 microspin column according to manufacturer's instructions (about 30 µl elution volume).
5. Count 1 µl of labeled oligonucleotide (50,000 to 100,000 cpm)

B. Annealing to the Complementary Strand
1. Add 10 µl (100 pmole) of the Complementary Strand to the γ²³P-labeled First Strand oligonucleotide.
2. Anneal strands by incubation: 1 minute at 95 °C, then 5 minutes at 37 °C followed by 30 minutes at room temperature.
3. Store labeled substrate at −20 °C in a box designed to block radiation from β-emitters.

C. Reaction Procedure
1. Prepare 1.25x Reaction Mix for 10 reactions:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per 10 reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x Reaction Buffer</td>
<td>10 µl</td>
</tr>
<tr>
<td>γ²³P-labeled Substrate</td>
<td>2 µl (5 pmole)</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>68 µl</td>
</tr>
</tbody>
</table>

2. Dilute MPG enzyme to 10, 25, 50 and 100 µg/ml with Enzyme Dilution Buffer. For a control, use the Enzyme Dilution Buffer alone.
3. Dispense 8 µl of 1.25x Reaction Mix into each tube.
4. Start each reaction by the addition of 2 µl of the appropriate diluted enzyme sample at 20 second intervals.
5. Incubate for 10 minutes at 37 °C.
6. Stop reactions by the addition 2.5 µl of 0.5 M 1,4-diaminobutane, pH 8.0
7. Boil for 5 min at 95 °C.
8. Add 5 µl of stop solution
9. Boil for 5 min at 95 °C

D. Gel Electrophoresis Analysis
1. Prepare 20% denaturing gel containing 7 M urea, assemble the electrophoresis apparatus, and add running buffer.
2. Pre-run the denaturing gel for 30 minutes at 100V, with circulating cold water to reduce heating.
3. Load 5 to 7 µl of each sample on the denaturing gel. Note: Wash the wells before loading.
4. Run the gel at 100 to 200 V, (bromophenol blue and xylene cyanole FF run as approximately 8 and 23 base oligonucleotides, respectively, on 20% denaturing gels).
5. 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.
6. Expose to X-ray film for 1 to 3 hours at −20 °C. It is recommended to put two layers of film on the gel in order to get at least one film properly exposed.
7. Develop the film and analyze the results.

References