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ProductInformation

Methylpurine DNA N-Glycosylase
Human, Recombinant

Product Number **M 7940**
Storage Temperature –70 °C

Product Description

Human methylpurine DNA N-glycosylase[1] (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 site. MPG recognizes and excises 3-methyladenine, 7-methylguanine, 3-methylguanine, N-6-ethanol-adenine and hypoxanthine.[2]

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 ^{32}P-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’ TTGGTTGGGTGGTHGGTGTTGGGG 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
- γ^{32}P-ATP 10 mCi/ml
- 10x Reaction Buffer: Prepare 10 ml of 500 mM HEPES, pH 7.5, containing 100 mM $MgCl_2$, 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:

Component	Volume
10x PNK Buffer	3 µl
First Strand Oligonucleotide	10 µl (100 pmole)
γ ^{32}P-ATP 10 mCi/ml	3 µl (30 µCi)
T4 PNK	1 µl
Deionized Water	13 µl (30 µl total)

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 ^{32}P-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:

Component	Volume per 10 reactions
10x Reaction Buffer	10 µl
^{32}P-labeled Substrate	2 µl (5 pmole)
Deionized Water	68 µl

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

1. Kreklau, E.L., Nucleic acid Res., **29**, 2558-2566 (2001).
2. Asaeda, A., Biochemistry, **39**, 1959-1965 (2002).
3. Current Protocols in Molecular Biology, Wiley, 2.12

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