SIGMA

# ProductInformation

**$O^6$-Methylguanine-DNA Methyltransferase**
**Human, recombinant**
expressed in *E. coli*

Product Number **M 8065**
Storage Temperature –20 °C

EC# 2.1.1.63
Synonyms: MGMT; AGT

## Product Description

$O^6$-Methylguanine-DNA Methyltransferase (MGMT) is a human recombinant protein expressed in *E. coli.* It contains 207 amino acids with a molecular weight of approximately 21 kDa (apparent MW by SDS-PAGE is 25 kDa).

MGMT is a ubiquitous DNA repair protein that removes $O^6$-alkyl-guanine, primarily $O^6$-methyl-guanine, lesions from damaged DNA. It is a major contributor to cellular protection from the mutagenic, carcinogenic, and cytotoxic effects of DNA alkylation. The mechanism of MGMT action is based on the transfer of the alkyl group from the DNA to a unique acceptor cysteine residue in the protein, forming a stable thioether linkage. The cysteine sulfhydryl moiety is not regenerated and therefore, MGMT is frequently classified as a suicide protein. Since the reaction irreversibly inactivates the enzyme, the repair capacity for $O^6$-methylguanine is dependent on the number of MGMT molecules in the cell. There is a correlation between the occurrence of cancer in various tissues and the lack of the MGMT enzyme. High levels of MGMT result in reduced tumor events and resistance of tumors to alkylating agents.

This product is supplied as a solution in 50% (w/v) glycerol containing 20 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, and 200 mM NaCl.

Purity: minimum 90% (SDS-PAGE)

Specific Activity: minimum 10,000 units per mg protein

Unit Definition: The amount of protein that removes 50% of the methyl groups of 0.5 pmol double stranded DNA oligonucleotide substrate containing an $O^6$-methylguanine 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 wet ice and storage at –20 °C is recommended. Do not allow the solution to freeze.

## Procedure

The activity of MGMT is determined by measuring the extent of demethylation of DNA containing $O^6$-methyl-guanine. For the assay of this enzyme, a 23 base oligonucleotide containing $O^6$-methylguanine at the eighth base position, which is within a unique *Pst*I restriction site, is $^{32}P$ labeled by polynucleotide kinase (PNK) and annealed to a complementary strand. Following incubation with MGMT, which removes the methyl group from $O^6$-methylguanine, the substrate is incubated with the *Pst*I restriction enzyme, which cuts the repaired substrate into 8 and 15 base oligonucleotides. Denaturation of the double stranded oligonucleotides and separation on a denaturing (7 M urea) PAGE gel results in the appearance of a 8 base labeled band in addition to the original 23 base band. The substrate used in this assay is:

First strand:
GAACTLCAGCTCCGTGCTGGCCC (first strand)
L = $O^6$-Me-dG

Complimentary strand:
GGGCCAGCAGGGAGCTGCAGTTC

Reagents and Equipment

- MGMT
- T4 polynucleotide kinase (PNK) (Product No. P 4390).
- T4 polynucleotide kinase (PNK) buffer.
- $\gamma\,^{32}P$-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
- *Pst*I restriction enzyme (20,000 units/ml)
- 10x *Pst*I buffer

- Stop Solution: 90% formamide, 0.1% (w/v) bromophenol blue, 0.1% (w/v) xylene cyanole FF, and 20 mM EDTA.
- Desalting column (G-25 microspin column) for 50 μl sample.
- 20% denaturing (7 M urea) acrylamide gel and electrophoresis apparatus.
- TBE gel running buffer (Product No. T 9525)
- X-ray film and developing machine

Preparation of double stranded oligonucleotide substrate

A. $^{32}P$ labeling of the First Strand oligonucleotide

1. Prepare the following mix:

| **Component** | **Volume** |
|---|---|
| 10x PNK buffer | 3 μl |
| First Strand oligonucleotide | 10 μl (100 pmole) |
| $\gamma$ $^{32}P$-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 13 μl (130 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 radioactive protected box.

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 MGMT 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. Incubate for 5 minutes at 65 °C (to stop the activity of MGMT).
7. Add 1μl of *Pst*I and 1μl of the 10x *Pst*I buffer to each tube.
8. Incubate for 60 minutes at 37 °C.
9. Stop reactions by the addition of 5 μl of the Stop Solution to each tube.
10. Heat at 95°C for 5 minutes, then keep on ice.

Gel Electrophoresis Analysis

1. Prepare 20% denaturing gel containing 7 M urea, assemble the electrophoresis apparatus, and add running buffer.[7]
2. Pre-run the denaturing gel for 30 minutes at 100 V, 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**

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