ProductInformation

MutY
from *E.Coli*, recombinant
Expressed in *E. Coli*

Product Code **M 1188**
Storage Temperature –20 °C

Synonyms: Adenine glycosylase MutY

Product Description
MutY is an *E. Coli* recombinant protein overexpressed in *E. coli* as the fully active enzyme. MutY, a 39 kDa protein, consists of a large N-terminal domain of 26 kDa and a small C-terminal domain of 13 kDa. The large domain contains the DNA binding activity and the catalytic activity of the intact MutY.[1] The cysteine bound iron renders the protein a light green color.

The adenine glycosylase MutY from *E. Coli* represents a subclass of BER (base excision repair) enzymes, which participate in the repair of oxidative DNA damage, and contains an $[Fe_4S_4]^{2+}$ center similar to that found in endonuclease III. It's most important substrate is the A:8-oxo-G mispair, where the base removed is the undamaged adenine leaving an abasic site and an intact phosphodiester backbone.[2-5]

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

Purity: minimum 90% (SDS-PAGE)

Specific Activity: minimum 20,000 units per mg protein

Unit Definition: The amount of protein that cleaves 50% of 0.5 pmole of double stranded DNA oligomer substrate containing an dA:8-oxo-dG 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 wet ice and storage at –20 °C is recommended. Do not allow the solution to freeze.

Procedure
MutY is an adenine DNA glycosylase that acts preferentially on the A:8-oxo-G mismatch and removes the undamaged adenine from the double stranded DNA to create an abasic site. For the assay of this enzyme, a 23 base oligonucleotide containing A at the eleventh base position is ^{32}P labeled by polynucleotide kinase (PNK) and annealed to a complementary strand having 8-oxo-G opposite the adenine. During the reaction, MutY removes the adenine from the labeled first strand and the abasic site is then nicked when putrescine is added to the reaction mix. Denaturation of the double stranded oligonucleotides and separation on a denaturing (7 M urea) PAGE gel results in the appearance of a 10 base labeled band in addition to the original 23 base band. The substrate used in this assay is:

First Strand
5'-CTCTCCCTTCACTCCTTTCCTCT-3'
Complementary Strand
5'-AGAGGAAAGGAG(8-oxo-G)GAAGGGAGAG-3'

Reagents and Equipment
- MutY
- 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 Tris-HCl, pH 7.6, containing 20 mM EDTA and 500 mM KCl.
- 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
- 0.5 M putrescine, pH 8 (Product No. P 7630).
- 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 (dA) oligonucleotide	10 µl (100 pmole)
ATP γ-^{32}P 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 label oligonucleotide (50,000 to 100,000 cpm)

B. Annealing to the Complementary Strand

1. Add 13 µl (130 pmole) of the complementary strand (8-oxo-G) 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 20% denaturing gel containing 7 M urea, assemble the electrophoresis apparatus, and add running buffer.[6]
2. Prepare reaction mix for 10 reactions:

Component	Volume per 10 reactions
10x Reaction Buffer	10 µl
^{32}P labeled substrate	2 µl (~5 pmole)
Distilled water	68 µl

3. Dilute the MutY enzyme to 1, 5, 10, and 20 µg/ml with enzyme dilution buffer.
4. Dispense 8 µl of reaction mix into each tube.
5. Start each reaction by the addition of 2 µl of the appropriate diluted enzyme sample at 20 second intervals. For a control add 2 µl of enzyme dilution buffer in place of the enzyme to one sample.
6. Incubate for 10 minutes at 37 ºC.
7. Add 2.5 µl of 0.5 M putrescine, pH8, to each reaction.
8. Boil each reaction for 5 minutes at 95 ºC.
9. Stop reactions by the addition of 5 µl of the stop solution.
10. Boil for 5 minutes at 95 ºC, then keep on ice.
11. Pre-run the gel for 30 minutes at 100 V, with circulating cold water to reduce heating.
12. Load 4 to 6 µl sample on 20% denaturing gel. Note: Wash the wells before loading.
13. Run the gel at 150 V with circulating cold water (~10 ºC) to reduce heating until the stain front reaches 1 to 2 cm from the bottom of the gel (bromophenol blue and xylene cyanole FF run as an 8 base and 28 base oligonucleotides, respectively, on 20 % denaturing gels).
14. 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.
15. Expose to X-ray film for 16 hours at –20 ºC. It is recommended to put two layers of film on the gel in order to get at least one film properly exposed.

References

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3. Golinellli M. P. et al., Biochemistry, **38**, 6997-7007 (1999).
4. Chepanoske, C. L. et al., Nucleic Acids Res., **27**, 3197-3204 (1999).
5. Zharkov, D. O., and Grollman, A.P., Biochemistry, **37**, 12384-12394 (1998).
6. Current Protocols in Molecular Biology, Wiley, 2.12

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