ProductInformation

8-Oxoguanine-DNA glycosylase (OGG1)
Murine, Recombinant

Product Number O 2135
Storage Temperature –20 °C

Product Description
This product is a recombinant murine protein expressed in *E. coli*.

8-Oxoguanine-DNA glyosylase (OGG1) is a DNA repair protein that is primarily responsible for the repair of 8-oxoguanine (8-oxo-G). It possesses both DNA glycosylase and AP (apurinic) lyase activity and is a functional analog of the bacteria Fpg protein. OGG1 releases 8-oxoguanine (8-oxo-G) from mutated DNA and nicks the DNA strand.[1-2] Although its glycosidase activity is very efficient, its lyase activity is weak and is highest for substrates having a C as the opposite base.[3] OGG1 is a β-lyase generating an α,β-unsaturated sugar moiety at the 3'-terminus.[4] Murine OGG1 is a 345 amino acid protein of 38.8 kDa. It displays 84% identity to the human α-OGG1.[2] The protein is expressed in *E. coli* and its activity is determined on a double-stranded oligonucleotide containing 8-oxoguanine having a C opposite the lesion.[4]

Vial content: 10 µg of protein (Bradford) in 50% glycerol containing 20 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, and 200 mM NaCl.

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

Unit definition: One unit is the amount of protein that cleaves 50% of 0.5 pmol of double strand DNA oligonucleotide substrate (8-oxoguanine mutated) in 10 minutes at 37 °C.

Storage/Stability
Store at –20 °C. **Do not freeze at –70 °C.**

Preparation Instructions
Reagents
1. ^{32}P labeled OGG1 substrate ds-oligonucleotide: Prepare the substrate according to the technical bulletin for the Fpg Protein Substrate Set, (Product Code F 9550).
2. 10X Reaction Buffer: 500 mM Tris-HCl, pH 7.6, 20 mM EDTA, and 500 mM KCl.
3. Enzyme Dilution Buffer**:** 50 mM K-HEPES, pH 7.6, 1 mM EDTA, 1 mM DTT, 10% (v/v) glycerol, and 200 mM NaCl.
4. Stop Solution: 90% (v/v) formamide, 0.1% (w/v) Bromphenol Blue, 0.1% (w/v) Xylene Cyanole FF, and 20 mM EDTA.
5. TBE Running Buffer: 89 mM Tris base, 2 mM EDTA, and 89 mM boric acid, pH 8.0.
6. Putrescine Solution: 0.5 M putrescine free base, pH adjusted to 8 with HCl
7. Also required: 20% denaturing gel, X-ray film, and developing machine

Procedure
The OGG1 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-stranded DNA. The substrate used for assaying the activity of OGG1 protein is a radiolabeled ds-23 oligonucleotide containing 8-oxo-dG at the eleventh base of the labeled strand (Product Number F 9550, the OGG1 substrate used is identical to the Fpg substrate.).

In the reaction, OGG1 first removes the 8-oxo-G base and then cleaves the mutated strand at the apurinic site. Putrescine is added because OGG1 does not produce nicks in DNA very efficiently.[3] Denaturation of the double-stranded residual substrate and cleavage product results in single-stranded oligonucleotides. These are separated on a denatured polyacrylamide gel, producing ^{32}P labeled bands. The cleavage product combines bands from β and δ elimination.

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

Component	Total volume for 10 runs
10X Reaction Buffer	10 µl
^{32}P labeled OGG1 substrate	2 µl (~5 pmol)
Distilled water	68 µl

3. Dilute OGG1 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 of diluted enzyme sample per tube using 20 second intervals. For a control, use 2 µl of the Enzyme Dilution Buffer in one reaction.
6. Incubate for 10 minutes at 37 °C.
7. Add 2.5 µl of the Putrescine Solution.
8. Heat for 5 minutes at 95 °C.
9. Stop reactions by the addition of 5 µl of the Stop Solution.
10. Heat for 5 minutes at 95 °C.
11. Pre-run the gel for 30 minutes at 100 Volts with circulating cold water to reduce heating.
12. Load 4-6 µl of the reaction mixture on the 20% denaturing gel.
    Note: wash the wells before loading.
13. Run the gel at 150 Volts with circulating cold water (~10 °C) to reduce heating, until the leading dye reaches 1-2 cm from the bottom of the gel (Bromphenol Blue and Xylene Cyanole FF run as 8 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 as it may crack.
15. 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 sheet of film properly exposed.

References

1. Laval, J. et al., Mutation Res., **402**, 93-102 (1998).
2. Boiteux, S., and Radicella, P.J., Arch. Biochem. Biophys., **377**, 1-8 (2000).
3. Zharkov, D.O. et al., J. Biol. Chem., **275**, 28607-28617 (2000).
4. Rosenquist, T.A., Proc. Natl, Acad, Sci, USA, **94**, 7429-7434 (1997)

MAM 5/01