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ProductInformation

Fpg Protein SUBSTRATE SET

Product Number **F 9550**
Storage Temperature –20 °C

Product Description

This set contains a 23 base 8-oxo-G mutated oligonucleotide and a 23 base normal complementary oligonucleotide necessary to produce a radiolabeled ds-oligonucleotide substrate for Fpg Protein.

Specifically mutated double stranded (ds) oligonucleotides are replacing irradiated or chemically oxidized DNA as substrates for DNA repair proteins. The reason for this switch is the simplicity of mutated ss-oligonucleotide preparation by automated oligonucleotide synthesizers, the specificity of the assay and the simplicity of the detection methods.[1]

The procedure requires radioactive labeling of the oligonucleotide substrate. The radiolabeled ds-oligonucleotide substrate is prepared by 5'- ^{32}P labeling of the 8-oxo-G mutated strand followed by annealing to the complementary strand. The DNA repair enzymes (e.g. Fpg, Ogg1) are able to cleave the ds-oligonucleotide substrate at the mutated nucleotide, which is located at the middle of the labeled strand.[2,3] The fragments obtained are denatured and separated on an acrylamide-urea gel, which is then exposed to X-ray film.

Typically one preparation of the ^{32}P radiolabeled substrate is made with 100 pmol of mutated oligonucleotide strands and 130 pmol of complementary oligonucleotide strands. This preparation is sufficient for approximately 100 enzymatic tests. The substrate radioactivity decays with time ($t_{1/2}$= 14 days). ^{32}P radiolabeled substrate may be used for a maximum of 4 weeks.

The Fpg Protein Substrate Set contains sufficient oligonucleotides for 3 preparations of radiolabeled ds-oligonucleotide (100 enzymatic tests each).

Components

F 9300 Fpg Protein Substrate, 8-oxo-G Strand, vacuum dried, 350 pmol. **1 vial**.
23 bases, 8-oxo-G at position 11

F 9425 Fpg Protein Substrate Complementary Strand, vacuum dried 450 pmol. **1 vial**.
23 bases

Storage/Stability

Store dessicated at –20 °C.

Reconstitution Instructions

1. Reconstitute Fpg Protein Substrate 8-oxo-G Strand (Product Code: F 9300) with 35 µl molecular biology grade deionized water. Store at –20 °C.
2. Reconstitute Fpg Protein Substrate Comp. Strand (Product Code: F 9425) with 45 µl molecular biology grade deionized water. Store at –20 °C.

<u>Equipment and Reagents Needed but not Provided</u>

- Fpg Protein (Product Code F 3174) or other DNA repair enzyme.
- T4 polynucleotide kinase (PNK) (Product Code P4390).
- T4 polynucleotide kinase (PNK) buffer .
- γ ^{32}P-ATP 10 mCi/ml
- Fpg Protein dilution buffer:
  50 mM HEPES, pH 7.6, 10% glycerol, 250 mM NaCl, 1 mM EDTA, 1 mM DTT.
- 10X Reaction buffer:
  0.5 M Tris, pH 7.5, 0.5 M KCl, 20 mM EDTA.
- Stop solution:
  90 % formamide, 0.1 % w/v bromophenol blue, 0.1 % xylene cyanole, 20 mM EDTA, pH 8.
- G-25 microspin column.
- 20% denatured (7M urea) acrylamide gel and electrophoresis apparatus.
- TBE Running Buffer:
  89mM Tris, 2mM EDTA, 89mm Boric acid pH 8.0
- X-ray film and developing machine.

Procedure

Principle of Assay
The assay is based on the ability of DNA repair proteins such as Fpg Protein to cleave 8-oxo-G mutated double strand oligonucleotide. The 8-oxo-G strand is first labeled with 5' ^{32}P and then annealed to its complementary strand to form the Fpg Protein Substrate.

Fpg Protein recognizes and removes the mutated base (8-oxo-G), then cleaves the DNA at it's apurinic (AP) site (lyase activity). Following denaturation, a 10 bp band appears (in addition to the 23 bp band) on a denaturing (7 M urea) 20% PAGE.

Note: Use molecular biology grade water

Radiolabeled Substrate Preparation

Labeling of 8-oxo-G Mutated Strand
1. Prepare the following mix:

Reagent	
10X PNK buffer	3 µl
8-oxo-G strand oligonucleotide	10 µl (100 pmol)
ATP γ-^{32}P 10 mCi/ml	3 µl (30 µCi)
T4 PNK	1 µl
Deionized H_2O	13 µl (30 µl total)

2. Incubate for 60 min at 37 °C.
3. Inactivate for 10 min at 70 °C.
4. Clean sample from remaining ATP on G-25 microspin column according to manufacturer instructions (about 30 µl elution).

Annealing to the Complementary Strand
1. Add 13 µl (130 pmol) of complementary strand (Product Code: F 9425).
2. Anneal strands by incubation: 1 min. at 95 °C and 5 min. at 37 °C and then 30 min. at room temperature.
3. Store labeled substrate at –20 °C in a radioactive protected box.

Enzymatic Assay Procedure
1. Prepare 20% denaturing gel (7 M urea) and assemble the electrophoresis apparatus.
2. Dilute Fpg Protein enzyme using the dilution buffer.
3. Prepare reaction mix for 10 reactions.

Reagent	Amount per 10 reactions
10X reaction buffer	10 µl
Labeled Fpg Protein substrate	2 µl
Deionized H_2O	68 µl

4. Dispense 8 µl of reaction mix to each tube.
5. Start the reaction by the addition of 2 µl diluted enzyme samples with 20 seconds intervals. For control add 2 µl of dilution buffer in place of enzyme.
6. Incubate for 10 min. at 25 °C.
7. Stop reactions by the addition 5 µl stop solution.
8. Boil for 5 min. at 95 °C.
9. Load 4 µl sample on the denaturing gel. Note: wash the wells before loading.
10. Run the mini gel at 200V with circulating cold water (~10°C) to reduce heating until the stain front reaches 1-2 cm of the bottom of the gel (bromophenol blue and xylene cyanole run as 8 and 28 bases respectively on 20% denaturing gels).
11. Carefully disassemble the gel and lay it on a piece of Whatman 3 mm paper.
12. Cover the gel with a sheet of plastic wrap. Note: do not dry the gel, it may crack.
13. 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 gel properly exposed.

References

1. Tchou, J., et al., J. Biol. Chem., **269**, 5318-15324 (1994).
2. Chung, M.H., et al., Mutation Res., **254**, 1-12 (1991).
3. Tchou, J., et al., Proc. Natl. Acad. Sci., **88**, 4690-4694 (1991).
4. Current Protocols in Molecular Biology, Wiley, 2.12.

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