Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Endonuclease VIII
(Endo VIII, nei Protein)
E. coli, Recombinant

Product Number **E 0651**
Storage Temperature –20 °C

Product Description

This product is an 263 amino acid (30 kDa) *E. coli* recombinant protein over-expressed in *E.coli.*

Endonuclese VIII (endo VIII) from *E.coli*, a product of the *nei* gene, is a DNA repair protein that recognizes and removes modified pyrimidines, such as thymine glycol (Tg) and 5,6, dihydrothymine (DHT) from DNA.[1] Endo VIII possesses both DNA glycosylase and apurinic/apyrimidinic (AP) lyase activities. Its modified-base substrate specificity overlaps the endonuclease III (endo III, *nth*) substrate specificity. This cross reactivity is manifested in *E.coli* mutants. While *E.coli nth* and *nei* mutants are not sensitive or slightly more sensitive to ionizing radiation and hydrogen peroxides than wild type, the *nei-nth* double mutant is hypersensitive to oxidative stress.[2,3] The mechanism used by Endo VIII to cleave the DNA backbone (lyase activity) is β-δ elimination, resembling the action of fpg protein and not endo III, which cleaves the DNA backbone by β elimination.[3,4]

Activity is measured using a 5,6,-dihydrothymine (DHT)-mutated ds-oligonucleotide.[4]

Specific activity: >20,000 units per mg protein.

Unit definition: The amount of protein that cleaves 50 % of 0.5 pmol double stranded DNA oligonucleotide substrate containing DHT in 10 min at pH 7.6 at 30 °C.

Purity: > 90 % by SDS-PAGE.

Reagents

Vial content: A solution in 10 % glycerol containing 20 mM Tris pH 7.4, 100 mM NaCl, 0.5 mM EDTA, 1 mM DTT.

Storage/Stability

Store at –20 °C.

Procedure

Reagents & Supplies Required but not Supplied

-
- **^{32}P labeled Endonuclease VIII and III substrate ds-oligonucleotide** . Prepare the substrate according the product information sheet for the Endonuclease VIII & III Substrate Set, (Product Number E 7651).
- **10X reaction buffer**: 500 mM Tris, pH 7.6, 20 mM EDTA, 500 mM KCl.
- **Enzyme dilution buffer**: 20 mM Tris, HCl, pH 7.4, 0.5 mM EDTA, 1 mM DTT, 10 % v/v glycerol, 100 mM NaCl.
- **Stop solution**: 90 % formamide, 0.1 % (w/v) bromophenol blue, 0.1 % (w/v) xylene cyanole FF, 20 mM EDTA.
- 20 % denaturing gel.
- **TBE Running buffer**: 89 mM Tris base, 2 mM EDTA, 89 mM Boric acid, pH 8.0.
- X-ray film and developing machine

Principle of Assay
The Endo nuclease VIII activity assay is based on its glycosylase activity that recognizes and removes the mutated base 5,6-DHT which is followed by its lyase activity that cleaves the AP (apurinic/apyrimidinic) strand of the double strand DNA. The substrate used for assaying the activity of Endonuclease VIII protein is a radiolabeled ds-33 oligonucleotide containing DHT at the 16th base of the labeled strand (Product Number: E 7651, Endonuclease VIII and III Substrate Set).

In the reaction, Endonuclease VIII first removes the 5,6-DHT and then cleaves the mutated strand at the apyrimidinic site. Denaturation of the double strands and separation on denatured polyacrylamide gel produce two labeled bands: a 33 bp oligonucleotide band (residual uncleaved substrate) and a 15 bp nucleotide band (the cleavage product).

Assay

1. Prepare 20 % denaturing gel containing 7 M urea, assemble the electrophoresis apparatus and add running buffer.[5]
2. Prepare reaction mix for 10 reactions:

Reagent	Amount per 10 reactions
10X Reaction Buffer	10 µl
^{32}**P** labeled substrate	2 µl (~5 pmol)
Distilled water	68 µl

1. Dilute Endo VIII enzyme to 1 µg/ml, 5 µg/ml, 10 µg/ml, and 20 µg/ml with enzyme dilution buffer.
2. Dispense 8 µl of reaction mix to each tube
3. Start the reaction by the addition of 2 µl diluted enzyme samples using 20-second intervals. For a control add 2 µl of enzyme dilution buffer in place of the enzyme to one sample.
4. Incubate for 10 min. at 25 °C.
5. Stop reactions by the addition of 5 µl stop solution.
6. Boil for 5 min. at 95 °C then keep on ice.
7. Pre-run the gel for 30 min. at 100 Volts, with circulating cold water to reduce heating.
8. Load 4 µl sample on 20 % denaturing gel. Note: wash the wells before loading.
9. Run the gel at 150 V with circulating cold water (~10 °C) to reduce heating until the stain front reaches 1 cm to 2 cm from the bottom of the gel (bromophenol blue and xylene cyanole run as an 8 base and 28 base oligonucleotide on 20 % denaturing gels).
10. 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.
11. 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. Laval, J., et al., Mutation Res., **402**, 93-102 (1998).
2. Jiang, D., et al., J. Bacteriol., **179**, 3773-3782 (1997).
3. Jiang, D., et al., J. Biol. Chem., **272**, 32230-32239 (1997).
4. D'Ham, C., et al., Biochemistry, **38**, 3335-3344 (1999).
5. Current Protocols in Molecular Biology, Wiley, 2.12.

lpg 03/02