TREVIGEN® Product Data

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E. coli Endonuclease VIII

Catalog #: 4060-01K-EB

Contents: 4060-01K-01 Endonuclease VIII — **Size:** 1000 Units
3900-500-03 10X REC™ Buffer 3 — 1.0 ml

Description: Endonuclease VIII is an *N*-glycosylase with an associated Class I AP lyase activity, specific for a number of modified bases including β-ureidoisobutyric acid and DNA containing urea. Endonuclease VIII is similar in most repair capacities to Endonuclease III, except it has β, and δ lyase activity rather than only β lyase activity. All of the Endonuclease III substrates tested to date are also substrates for Endonuclease VIII.

Source: Purified from *E. coli* containing a recombinant plasmid harboring the *E. coli* nei gene.

Unit Definition: One unit is the amount of enzyme required to cleave an AP site oligonucleotides within an oligonucleotide duplex at the rate of 1 pmol/hour at 37°C.

Substrate Specificity: The enzyme recognizes substrates such as cis- and trans-thymine glycol, urea, 5-hydroxy-5-methylhydantion, and 5-hydroxy-6-hydrouracil.

Assay Conditions & Analysis: 1X REC Buffer 3 (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, and 1 mM EDTA), 1 pmole of an AP-site Oligonucleotide labeled with ^{32}P, 1 pmole of complementary oligonucleotide, and serial dilutions of enzyme in a 20 µl reaction volume are incubated for 1 hour at 37°C. Cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. The bands are cut out and radioactivity is counted to quantify the cleavage products.

Storage Buffer: 20 mM HEPES-KOH (pH 7.0), 50 mM NaCl, 1 mM EDTA, 0.1 mg/ml BSA, 0.1 % 2-mercaptoethanol, and 50% (v/v) glycerol.

Storage Conditions: Store at -20°C in a manual defrost freezer. For long term storage, freeze in working aliquots at - 80°C. Avoid repeated freeze-thawings.

References: 1. Wallace, S.S., H. Ide, Y.W. Kow, M.F. Laspia, R.J. Melameade, L.A. Petrullo, and E. LeClerc. 1998. Mechanisms and Consequences of DNA Damage Processing (Friedberg, E.C. and Hanawalt, P.C., eds.), pp. 151-157. New York:Alan R. Liss. 2. Melameade, R.J., Z. Hatahet, Y.W. Kow, H. Ide, and S.S. Wallace. 1994. Isolation and characterization of endonuclease VIII from Escherichia coli. Biochemistry **33**:1255-1264.

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E. coli
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