

TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

E. coli Endonuclease III (Thymine Glycol-DNA Glycosylase)

Catalog #: 4045-05K-EB

Contents: 4045-01K-01 Endonuclease III **Size:** 5 x 1000 Units
 3900-500-04 10X REC™ Buffer 4 1 ml

Description: Endonuclease III is a DNA glycosylase with an associated AP lyase activity. Endonuclease III releases bases damaged by UV light, ionizing radiation, osmium tetroxide, or acid. It cleaves abasic sites by β -elimination, producing a single nucleotide gap in the DNA, and contains an iron-sulfur group which helps to maintain its three dimensional conformation. The enzyme has a molecular weight of 23.4 kDa and is suitable for use in FLARE™.

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

Unit Definition: One Unit of enzyme cleaves 1 pmole of an oligonucleotide probe containing an AP site within an oligonucleotide duplex in one hour at 37 °C.

Substrate Specificity: Endonuclease III catalyzes the excision of the following forms of DNA damage: *Cis-trans*-thymine glycol, 5,6-dihydrothymine, 5,6-dihydroxydihydrothymine, alloxan, urea, uracil, 5-hydroxy-5-methylhydantoin, methyltartronylurea, 6-hydroxy-5,6-dihydro-pyrimidines, 5-hydroxycytosine, 5-hydroxyuracil, 5-hydroxy-6-hydrothymine, 5,6-dihydrouracil, glycol, 5-hydroxy-6-hydrouracil, and AP sites.

Assay Conditions & Analysis: Enzyme may be diluted in 10 mM HEPES-KOH (pH 7.4) and 100 mM KCl for immediate use. In 1X REC Buffer 4 (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 10 mM EDTA), add 4 pmole of a ³²P-labeled oligonucleotide containing an AP site, 4 pmole of a complementary oligonucleotide, and serial dilutions of enzyme in a 20 μ l reaction volume; incubate for 1 hour at 37 °C. The cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. Bands are cut out and radioactivity counted to quantify the cleavage products.

Storage Buffer: 10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 0.1 mg/ml BSA, and 50% (v/v) glycerol.

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

Reference:

Hatahet, Z., Y.W. Kow, A.A. Purnal, R.P. Cunningham, and S.S. Wallace. 1994. New substrates for old enzymes: 5-hydroxycytidine and 5-hydroxy-2'-deoxyuridine are substrates for *Escherichia coli* endonuclease III and formamidopyrimidine DNA-N glycosylase while 5-hydroxy-2'-deoxyuridine is a substrate for uracil DNA-glycosylase. *J Biol Chem* 69:18814-18820.

TREVIGEN®

8405 Helgerman Court, Gaithersburg, MD 20877 USA Voice: 1-800-TREVIGEN
 (1-800-873-8443) • 301-216-2800 Fax: 301-560-4973 • e-mail: info@trevigen.com •
 www.trevigen.com

***E. coli* Endonuclease III**

Catalog#: 4045-05K-EB

Storage: -20 °C

TREVIGEN®

1-800-873-8443

www.trevigen.com