

# TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

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

**Catalog #:** 4045-01K-EB

**Contents:** 4045-01K-01 Endonuclease III **Size:** 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  $\beta$ -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 <sup>32</sup>P-labeled oligonucleotide containing an AP site, 4 pmole of a complementary oligonucleotide, and serial dilutions of enzyme in a 20  $\mu$ 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®

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