

TREVIGEN® Product Data

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E. coli MutY DNA Glycosylase

Catalog #: 4000-500-EB

Contents: 4000-500-01 *E. coli* MutY DNA Glycosylase **Size:** 5000 Units
3900-500-04 10X REC™ Buffer 4 1 ml

Description: *E. coli* MutY acts together with Fpg to prevent the potentially mutagenic consequences of 8-oxo-dG lesions. 8-oxo-dG lesions escaping repair by Fpg frequently pair with A during DNA replication, producing an 8-oxo-dG:A mispair. MutY removes the A from this base pair to initiate base excision repair. In the absence of MutY, DNA replication after an 8-oxo-dG:A mismatch results in thymine incorporation opposite the adenine in one of the daughter strands, creating a fixed mutation. MutY has an associated AP lyase activity.

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

Unit Definition: One unit of enzyme cleaves 1 pmole of an oligonucleotide duplex containing an A/G mismatch in 1 hour at 37 °C. Only the strand with the A is cleaved.

Assay Conditions & Analysis: 8 pmoles of each oligo in an A/G mismatch oligonucleotide set with the A oligo end-labeled with γ -³²P, 1X REC Buffer 4 (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 10 mM EDTA), and serial dilutions of enzyme in a 20 μ l reaction volume are incubated for 1 hour at 37 °C. For analysis, 10 μ l of 3X REC Alkaline Loading Buffer (0.3 M NaOH, 97% formamide, and 0.2% bromophenol blue) are added, the samples are heated at 95 °C for 15 min then fast cooled to 4 °C, and the cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. The bands are cut out and radioactivity counted to quantify the cleavage products.

Storage Buffer: 10 mM HEPES-NaOH (pH 7.4), 50 mM KCl, 1 mM EDTA, 1 mM DTT, 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. Enzyme may be diluted in 10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 1 mM EDTA, 0.1 mg/ml BSA, and 50% glycerol and stored at -20 °C for 2 weeks of experimental use. Otherwise, dilute enzyme in 1X REC Buffer 4 for immediate use. MutY loses less than 10% of activity after 24 hours at 37 °C.

References:

1. Lu, A. and I. Hsu. 1992. Detection of single DNA base mutations with mismatch repair enzymes. *Genomics* **14**:249-255.
2. Friedberg, E.C., G.C. Walker, and W. Siede. 1995. DNA Repair and Mutagenesis. American Society of Microbiology. Washington, D.C: ASM Press.
3. Hsu, I., W.E. Highsmith, J. Xu, and D. Kong. 1998. Mismatch cleavage detects base deletion in cystic fibrosis gene. *Biotechniques* **25**:692-696.

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Concentration:
Units:
Specific Activity:

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