

E. coli* MutY DNA Glycosylase*Catalog #:** 4000-500-EB**Contents:** *E. coli* MutY DNA Glycosylase
10X REC™ Buffer 4**Size:** 500 units
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: 1X REC Buffer 4 (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 10 mM EDTA), 4 pmoles of the necessary oligos from the A/G Mismatch Mesophilic Oligonucleotide Set (Cat # 3800-100-A) with A oligo end-labeled with ³²P, and serial dilutions of enzyme in a 20 µL reaction volume are incubated for 1 hour at 37° C. For analysis, 10 µL of 3X Alkali Loading Buffer (0.3 M NaOH, 97% formamide, and 0.2% bromophenol blue) are added, the samples are heated at 95° C for 10 minutes then fast cooled to 2 - 8° 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-KOH (pH 7.4), 100 mM KCl, 1 mM EDTA, 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, aliquot and store at -80° C. Avoid repeated freeze-thaw cycles. Enzyme may be diluted in storage buffer (see above) 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.
 2. Friedberg, E.C., et al. (1995) *DNA Repair and Mutagenesis.* American Society of Microbiology. Washington, D.C.:ASM Press.
 3. Hsu, I., et al. (1998) *Mismatch cleavage detects base deletion in cystic fibrosis gene.* *Biotechniques* **25**:692.

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