Recombinant *E. coli* MutY with Buffer

Catalog Number: 4000-500-EB

PRODUCT DESCRIPTION

Recombinant *E. coli* MutY acts together with *E. coli* Formamidopyrimidine-DNA Glycosylase, or Fpg, to prevent the potentially mutagenic consequences of 8-oxo-dG lesions. 8-oxo-dG lesions escaping repair by Fpg frequently pair with Adenine (A) during DNA replication, producing an 8-oxo-dG:A mispair. Recombinant *E. coli* MutY removes the A from this base pair to initiate base excision repair. In the absence of the enzyme, 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. Recombinant *E. coli* MutY has an associated AP lyase activity.

SOURCE

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

MATERIALS PROVIDED

PART	PART #	AMOUNT PROVIDED	STORAGE
Recombinant *E. coli* MutY	4000-500-01	5000 Units	Store at ≤ -20 °C in a manual defrost freezer. For long term storage, freeze in working aliquots at ≤ -70 °C. Avoid repeated freeze-thaw cycles. Enzyme may be diluted in storage buffer containing 0.1 mg/mL BSA and stored at ≤ -20 °C for 2 weeks of experimental use.
10X REC™ Buffer 4	3900-500-4	1 mL	

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 AND ANALYSIS

Prepare 1X REC Buffer 4 by diluting 10X REC Buffer 1:10 in distilled water. Incubate 4 pmoles of A/G mismatch oligonucleotide set with the A oligo end-labeled, 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 µL reaction volume for 1 hour at 37 °C. To complete cleavage of a basic site, fresh NaOH is added to final concentration of 166 mM then heated for 15 minutes at 95 °C. For analysis, 24 µL of 2X Loading Buffer (20 mM EDTA, 97% formamide, and 0.2% bromophenol blue) is added, the samples are heated at 95 °C for 5 min then fast cooled to 2-8 °C, and the cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. The bands are analyzed to quantify the cleavage products.

STORAGE BUFFER

20 mM Tris pH 7.5, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, and 50% (v/v) Glycerol.

REFERENCES

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2. Friedberg, E.C. *et al.* (1995) DNA Repair and Mutagenesis. American Society of Microbiology. ASM Press.
3. Hsu, I. *et al.* (1998) Biotechniques **25**:692.

Global bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **USA** TEL 800 343 7475 **Canada** TEL 855 668 8722
Europe | Middle East | Africa TEL +44 (0)1235 529449 **China** TEL +86 (21) 52380373