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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:

E. coli MutY DNA Glycosylase
Catalog #: 4000-500-EB
Contents: E. coli MutY DNA Glycosylase
Size: 500 units

Reference:

For Research Use Only. Not For Use In Humans.