Human 8-oxoGuanine DNA Glycosylase (hOGG1)

Catalog #: 4130-100-EB

Contents: hOGG1 **Size:** 100 units
10X REC™ Buffer 6 1 mL

Description: Reactive oxygen species generated from such things as ionizing radiation, cellular metabolism, and chemical genotoxins cause the DNA adducts 7,8-dihydro-8-oxoguanine (8-oxoG) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FaPy). Human 8-oxoguanine DNA glycosylase (hOGG1) catalyzes the removal of the 8-oxoG and FaPy through cleavage of the DNA phosphodiester bond following Schiff base chemistry. hOGG1 does not recognize the C=O of 8-oxoG as expected, but rather recognizes a proton on N7 of the nucleotide. By mispairing with adenine during replication, 8-oxoG gives rise to G/C to T/A transversions, a frequent somatic mutation in human cancers. In contrast, a FaPy lesion leads to termination of replication and, therefore, is not considered a pre-mutagenic lesion.

Source: Purified from *E. coli* containing a recombinant plasmid harboring the hOGG1 gene.

Unit Definition: One unit of hOGG1 catalyzes the cleavage of 1 pmole of a ^{32}P-oligonucleotide probe in 1 hour at 37° C at an 8-oxoG/C base pair within an oligonucleotide duplex.

Substrate Specificity: The catalytic activity of hOGG1 is dependent upon the base the 8-oxoG is paired with in the order of C>T>G, A. hOGG1 is also catalytically active when 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FaPy) is paired with C. FaPy is only repaired when base paired to cytosine.

Assay Conditions & Analysis: 1X REC Buffer 6 (20 mM Tris-Cl (pH 8.0), 1 mM EDTA, 1 mM DTT, 100 µg/mL BSA), 4 pmoles of 8-oxodG Oligonucleotide (Cat.# 3850-100-OL) labeled with ^{32}P, 4 pmoles of Oligo Complement A (Cat.# 3850-100-OL),and serial dilutions of enzyme in a reaction volume of 20 µL are incubated for 1 hour at 37° C. For analysis, 10 µL of 3X Alkali Loading Buffer (300 mM NaOH, 97% formamide, and 0.2% bromophenol blue) are added, the samples are heated to 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 the radioactivity is counted to quantify the cleavage products.

Storage Buffer: 20 mM Tris-Cl (pH 7.8), 1 mM EDTA, 100 mM NaCl, 1 mM DTT, and 50% glycerol.

Storage Conditions: Store at -20° C. For long term storage, aliquot and store at -80° C. Avoid repeated freeze-thaw cycles.

References:

1. Bruner, S.D., *et al.* (2000) *Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA.* Nature **403**:859.
2. Boiteux, S. and J.P. Radicella (2000) *The human OGG1 gene: structure, functions, and its implications in the process of carcinogenesis.* Arch. Biochem. Biophys. **377**:1.

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