

Human 8-oxoGuanine DNA Glycosylase (hOGG1) Catalog #: 4130-100-EB

Contents:

4130-100-01 hOGG1

Size: 100 Units

3900-500-06 10X RECTM 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 *a-hOGG1* gene (nuclear protein).

Unit Definition:

One Unit cleaves 1 pmole of a labeled oligonucleotide probe containing 8-oxoG base paired with C within a duplex oligo.

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 FaPy is paired with C. FaPy is only repaired when base paired to cytosine.

Assay Conditions and 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 labeled 8-oxo-dG oligonucleotide annealed to the complement oligonucleotide, and serial dilutions of enzyme in a reaction volume of 20 µl are incubated for 1 hour at 37°C. For analysis, 20 µl of 2X Loading Buffer (20 mM EDTA, 95% formamide, and 0.13% bromophenol blue) are added, the samples are heated to 95°C for 10 min then fast cooled to 4°C, and the cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis, and percent cleavage quantified.

Storage Buffer: 20 mM Tris-Cl (pH 7.8), 1.0 mM DTT, 1 mM EDTA, 100 mM NaCl, 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 to avoid repeated freeze-thawing.

References:

1. Bruner, S.D., D.P.G. Norman, and G.L. Verdine. 2000. Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature* **403**:859-866.
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**:1-8.

GENTAUR