Thermostable TDG Protein (Thymine DNA Glycosylase)

Catalog #: 4070-500-EB

Contents: Thermostable TDG Protein — **Size:** 500 units
10X REC™ Buffer 4 — 1 mL

Description: TDG is a thermostable thymine DNA glycosylase from *Methanobacterium thermoautotrophicum*. The optimal temperature for the enzyme is 65° C. The enzyme lacks significant AP lyase or endonuclease activity. TDG works effectively in heteroduplex analysis to detect C to T transitions.

Source: Thermostable TDG is purified from *E. coli* containing a recombinant plasmid harboring the *Methanobacterium thermoautotrophicum* TDG gene.

Unit Definition: One unit is the amount of enzyme required to cleave 1 pmole of an oligonucleotide duplex containing a T/G mismatch in 1 hour at 65° C. Only the strand containing the T is cleaved.

Substrate Specificity: TDG enzyme recognizes T/G mismatches in duplex DNA and cleaves the strand with the T. The opposite strand is not cleaved. The enzyme also recognizes G/G mismatches if at least one nearest neighbor is an A or T and nicks one strand or the other. The enzyme exhibits poor AP lyase activity.

Assay Conditions & Analysis: 1X REC Buffer 4 (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, and 10 mM EDTA), and 4 pmoles of the necessary oligos from Cat.# 3810-100-T to create a T/G mismatch with the T oligonucleotide labeled with ^{32}P, and serial dilutions of enzyme in a 20 µL reaction volume are incubated for 1 hour at 65° 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 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 the radioactivity is 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 1X REC Buffer 4 for immediate use. TDG protein in storage buffer can survive for up to 24 hours at 37° C with less than 10% loss in activity.

References:

1. Horst, J.P. and H.J. Fritz (1996) *Counteracting the mutagenic effect of hydrolytic deamination of DNA 5-methylcytosine residues at high temperature: DNA mismatch N-glycosylase Mig. Myth of the thermophilic achaeon Methanobacterium thermoautotrophicum.* EMBO J. **15**:5459.
2. Begley, T.J., and R.P.C. Cunningham (1999) *Methanobacterium thermoautotrophicum thymine DNA mismatch glycosylase; conversion of an N-glycosylase to an AP lyase.* Protein Engineering **12**:333.
3. Bazar, L.S., *et al.* (1999) *Mutation detection identification DNA analysis system (MIDAS) for the detection of known mutations.* Electrophoresis **20**:1141.

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