CREATIVE BIOMART®
Microbe

Recombinant M. Thermoautotrophicus TDG Protein

SPECIFICATION

Cat.No.	TDG-01M
Species	Methanobacterium thermoautotrophicum
Product Name	Recombinant M. Thermoautotrophicus TDG Protein
Product Overview	Recombinant M. Thermoautotrophicus TDG without tag is purified from E. coli containing a recombinant plasmid harboring the Methanobacterium thermoautotrophicum TDG gene. The enzyme which optimal temperature is 65 centigrade 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.
Source	E. coli
Storage	Store at -20 centigrade in a manual defrost freezer. For long term storage, freeze in working aliquots at ≤ -70 centigrade. Avoid repeated freeze-thaw cycles. Enzyme may be diluted in 1X REC Buffer 4 for immediate use. In storage buffer, it is stable for up to 24 hours at 37 centigrade with less than 10% loss in activity.
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 centigrade. Only the strand containing the T is cleaved.
Usage	Prepare 1X REC Buffer 4 by diluting 10X REC Buffer 1:10 in distilled water. Incubate 4 pmoles of T/G mismatch oligonucleotide set with the T oligo end-labeled, 1X REC Buffer 4 (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, and 10 mM EDTA), and serial dilutions of enzyme in a 20 µL reaction volume for 1 hour at 65 centigrade. To complete cleavage of a basic site, fresh 1N NaOH is added to final concentration of 166 mM then heated for 15 minutes at 95 centigrade. For analysis, 24 µL of 2X Loading Buffer (20 mM EDTA, 95% formamide, and 0.13% bromophenol blue) are added, and the samples heated at 95 centigrade for 10 min then fast cooled to 2-8 centigrade. The cleavage products are resolved by 20% denaturing polyacrylamide gel

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	electrophoresis, and percent cleavage quantified.
Storage Buffer	25 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 50% (v/v) Glycerol.

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