

PRODUCT DESCRIPTION

Recombinant M. Thermoautotrophicus TDG is originally from Methanobacterium thermoautotrophicum. The optimal temperature for the enzyme is 65 °C. The enzyme lacks significant AP lyase or endonuclease activity. It works effectively in heteroduplex analysis to detect C to T transitions.

SOURCE

Recombinant M. Thermoautotrophicus TDG is purified from *E. coli* containing a recombinant plasmid harboring the Methanobacterium thermoautotrophicum TDG gene.

MATERIALS PROVIDED

PART	PART #	AMOUNT PROVIDED	STORAGE
Recombinant M. Thermoautotrophicus TDG	4070-500-01	500 Units	Store at -20 °C in a manual defrost freezer. For long term storage, freeze in working aliquots at ≤ -70 °C. 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 °C with less than 10% loss in activity.
10X REC™ Buffer 4	3900-500-4	1 mL	

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

Recombinant M. Thermoautotrophicus 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 AND ANALYSIS

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 °C. 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 °C. 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 °C for 10 min then fast cooled to 2-8 °C. The cleavage products are resolved by 20% denaturing polyacrylamide gel 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.

REFERENCES

1. Horst, J.P. and H.J. Fritz (1996) EMBO J. **15**:5459.
2. Begley, T.J. and R.P.C. Cunningham (1999) Protein Engineering **12**:333.