# TREVIGEN® Product Data

***For Research Use Only. Not For Use In Diagnostic Procedures***

## Thermostable TDG Protein (Thymine DNA Glycosylase)

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| **Catalog #:** | 4070-500-EB | | | |
| **Contents:** | 4070-500-01 | Thermostable TDG Protein | **Size:** | 500 Units |
| | 3900-500-4 | 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:** 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 are incubated for 1 hour at 65°C. To complete cleavage of abasic 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 4°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.

**Storage Conditions:** Store at -20°C in a manual defrost freezer. For long term storage, freeze in working aliquots at -80°C. Avoid repeated freeze-thawing. 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 archaeon *Methanobacterium thermoautotrophicum*. EMBO J **15**:5459-5469.
2. Begley, T.J., and R.P.C. Cunningham.1999. *Methanobacterium thermoformicicum* thymine DNA mismatch glycosylase; conversion of an N-glycosylase to an AP lyase. Protein Engineering **12**:333-340.



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## Related Products:

| Catalog# | Description | Size |
|---|---|---|
| 4020-100-EB | Human DNA Polymerase β | 100 U |
| 4025-100-EB | *E. coli* Uracil-N-Glycosylase (UNGase) | 100 U |
| 4040-100-EB | *E. coli* Formamidopyrimidine-DNA Glycosylase (Fpg) | 500 U |
| 4045-01K-EB | *E. coli* Endonuclease III (Thymine Glycol-DNA Glycosylase) | 1000 U |
| 4050-100-EB | *E. coli* Endonuclease IV (nfo protein) | 100 U |
| 4055-100-EB | T4 Endonuclease V (T4-Pyrimidine Dimer Glycosylase/T4-PDG) | $10^5$ U |
| 4065-100-EB | Chlorella Virus Pyrimidine Dimer Glycosylase (cv-PDG) | 1000 U |
| 4090-500-EB | Mouse 3-mA DNA Glycosylase (Aag protein) | 500 U |
| 4100-100-EB | *S. pombe* UVDE | 100 µl |
| 4110-01K-EB | Human Apurinic/Apyrimidinic Endonuclease (hAPE) | 1000 U |
| 4120-100-EB | Human FEN-1 (Flap Endonuclease) | 100 U |
| 4135-250-01 | Human Ku 70/80 Complex | 250 U |
| 4130-100-EB | Human 8-oxoGuanine DNA Glycosylase (hOGG1) | 100 U |

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