MUTANT ENDONUCLEASE V

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Catalog No. EMV001

Tma endoV M2-6 (mutant *Thermotoga maritima* endonuclease V)

BACKGROUND

Tma endoV M2-6 is an endonuclease having modified substrate specificity created by amino acid substitutions to Tma endoV (endonuclease V from thermophilic bacterium *Thermotoga maritima*). Tma endoV has a deoxyinosine 3'-endonuclease activity, i.e. it recognizes a hypoxanthine (the deoxyinosine base) and exhibits a nicking activity to hydrolyze the second phosphodiester bond to 3' the lesion base [1-4]. The wild-type endoV also shows nonspecific DNA cleaving activities, independent of the specific recognition of the lesion structure. Tma endoV M2-6 exhibits a significantly reduced nonspecific activity toward an intact DNA and retains the specific endonuclease activity against a deoxyinosine-containing DNA.

Product type	**Endonucelase V**
Source	**Purified from *Escherichia coli* cells carrying a mutant endonuclease V gene (*nfi* gene), which was cloned from *Thermotoga maritima* and introduced site-directed mutagenesis [5].**
Volume	**100ul**
Concentration	**1 Unit/ ul**
Buffer composition	**25 mM HEPES (pH7.4), 0.5 mM EDTA , 50 uM DTT, 25 mM NaCl, 250 mM imidazole, 50% (v/v) glycerol**
Supplied	**10x Reaction buffer 1 ml (100 mM HEPES (pH7.4), 50 mM $MgCl_2$, 10 mM DTT)**
Definition of Activity Unit	**1 Unit is defined as the amount of enzyme exhibiting a complete degradation of 1 ug of deoxyinosine-containing substrate DNA at 65°C for 30 min, in a reaction containing 5 mM $MgCl_2$, 1 mM DTT, and 10 mM HEPES buffer (pH7.4). The substrate DNA is a purified DNA fragment synthesized by DNA polymerase in the presence of equimolar mixture of dATP, dCTP, dGTP, dTTP, and dITP. The degradation of the DNA is observed as the result of agarose gel electrophoresis.**
Molecular Weigh	**28kDa (SDS-PAGE)**
Optimum pH	**7.4**
Optimum Temperature	**65°C**
Storage	**Store at -20°C**

References

1) Huang J, et al., *Biochemistry*, 40(30), 8738-8748 (2001).
2) Huang J, et al., *Biochemistry*, 41(26), 8342-8350 (2002).
3) Hitchcock TM, et al., *Nucleic Acids Res*, 32(13), 4071-4080 (2004).
4) Feng H, et al., *Biochemistry*, 44(34):11486-11495 (2005).
5) Nakashima K, Takano F, Matsuo K, Ohiso I, Highly specific recognition and cleavage of deoxyinosine-containing DNA by mutant *Thermotoga maritima* endonuclease V. The 32nd Annual Meeting of the Molecular Biology of Japan (MBSJ2009).

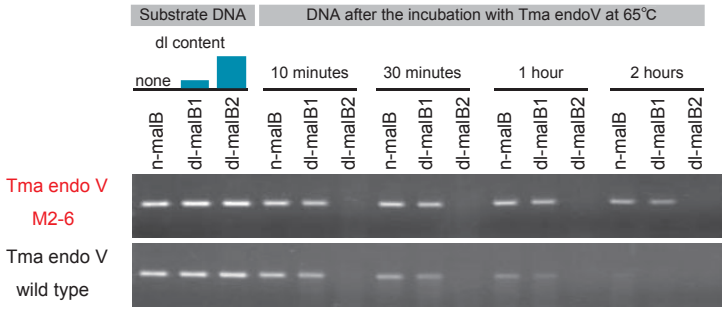

Figure 1. Degradation of double-stranded DNA with Tma endoV M2-6

Upper panel : 12 Units of Tma endoV M2-6 was incubated with 217 ng of double-stranded DNA containing various contents of dI in 1 x Reaction Buffer (total volume 50 µl) at 65 ℃ for indicated time. Lower panel : For comparison, the same amount of wild-type Tma endoV was incubated in the same conditions. Tma endoV M2-6 degraded only dI-containing DNA specifically, and exhibited no significant degradation of the normal DNA (not containing dI).

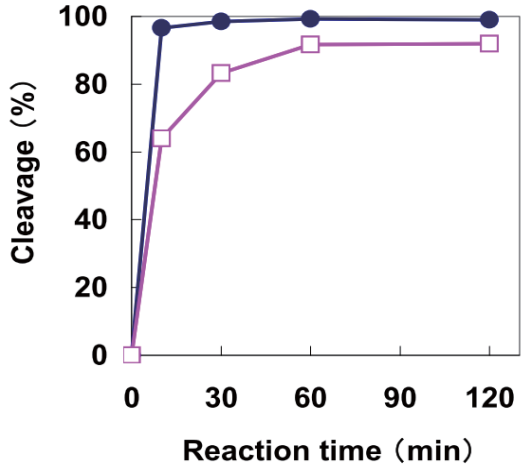

Figure 2. Cleavage time course of dI-containing double-stranded DNA.

1 Unit of Tma endoV M2-6 and 217 ng of dI-containing double-stranded DNA (dI-malB2) were incubated in 1 x Reaction Buffer (total volume 50 µl) at 65℃. Time courses of DNA cleavage (%) by the fresh enzyme (closed circles) and by the preheated enzyme at 65 ℃ for 1 hour (open boxes) are shown. Tma endoV M2-6 did not show significant loss of activity level even after the heat treatment at 65 ℃ for 1 hour.

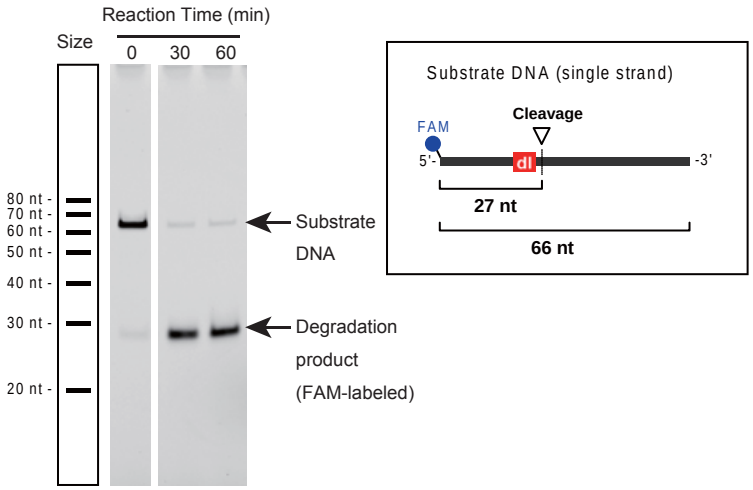

Figure 3. Degradation of single-stranded DNA with Tma endoV M2-6

Cleavage products were analyzed by denaturing polyacrylamide gel electrophoresis. The carboxyfluorescein(FAM)-labeled DNA fragments were excited at 473 nm.

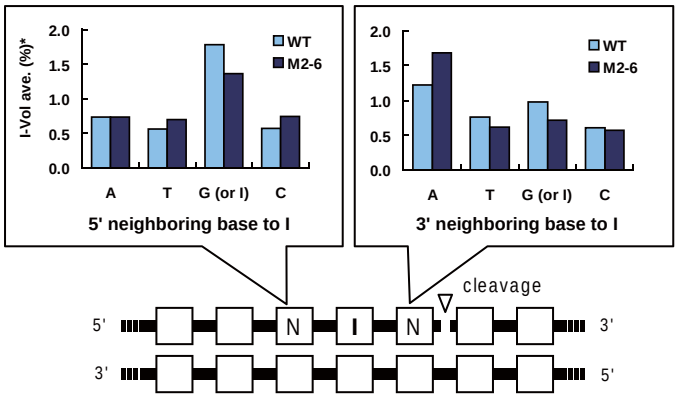

Figure 4. Relative preference of 5' or 3' neighboring base in dI recognition and cleavage by Tma endoV

* I-Vol ave. is the average signal intensity of each neighboring base to dI site for cleaved fragments from various sequence context.

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