DUPLEX-SPECIFIC NUCLEASE

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Catalog No. BTN102, BTN103

rTDSN (recombinant Taraba crab duplex-specific nuclease)

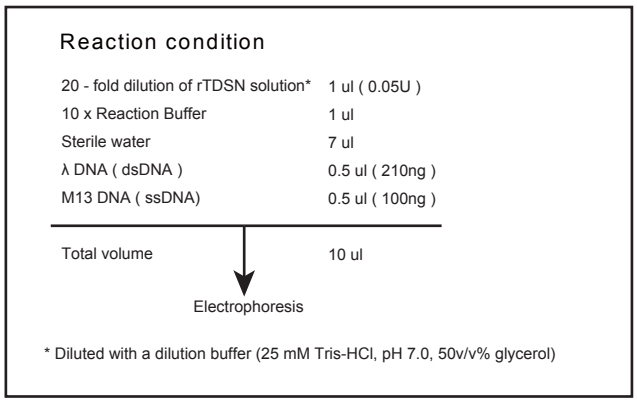
BACKGROUND

The rTDSN degrades double-stranded DNA and DNA in DNA-RNA hybrid duplex with high selectivity, while displays little cleaving activities to single-stranded DNA. The rTDSN is a thermostable enzyme with an optimum temperature around 65°C (maximum activity at 67°C). The rTDSN is tolerant to a proteinase K digestion. The duplex-specific nuclease activity is applicable to various purposes, for example, methods for SNP analysis [1], cDNA normalization [2], subtraction [3], and quantitative telomeric overhang determination [4].

Product type	**Duplex-specific nuclease**
Source	**Purified from insect cells infected by recombinant baculovirus containing *Paralithodes camtschaticus* (Taraba crab or red king crab) hepatopancreas cDNA for duplex-specific nuclease**[5, 6]**.**
Volume	**BTN102 : 50 ul, BTN103 : 100ul**
Concentration	**1 Unit/ ul**
Buffer composition	**25 mM Tris-HCl, pH 7.0, 50 v/v% glycerol**
Supplied	**10x Reaction buffer 1 ml (500mM Tris HCl, pH8.0, 70mM $MgCl_2$)**
Definition of Activity Unit	**1 Unit (Kunitz unit) is defined as the amount of enzyme causing an increase in absorption of 0.001 at 260 nm per minute at 25°C, in a solution containing 40 µg/ml calf thymus DNA, 7 mM $MgCl_2$, and 50 mM Tris-HCl buffer(pH 8.0). It is based on Kunitz method [Kunitz M., *J Gen Physiol*, 33, 349-362 (1950)].**
Molecular Weigh	**44 kDa (SDS-PAGE)**
Optimum pH	**6.5**
Optimum Temperature	**67°C**
Storage	**Store at -20°C**

References

1) Shagin DA, et al., *Genome Res*, 12(12), 1935-1942 (2002).
2) Zhulidov PA, et al., *Nucleic Acids Res*, 32(3), e37 (2004).
3) Peng RH, et al., *Anal Biochem*, 372(2), 148-155 (2008).
4) Zhao Y, et al., *Nucleic Acids Res*, 36(3), e14 (2008).
5) Hirakawa Y, Ohiso I, Expression of duplex-specific nuclease derived from *Paralithodes camtschaticus* by insect cells-baculovirus system. The 11th Annual Meeting of Japanese Society for Marine Biotechnology (2008).
6) Hirakawa Y, Ohiso I, Establishment of production strategy of recombinant red king crab duplex-specific nuclease using baculovirus expression system. Joint Annual Meeting of the Molecular Biology Society of Japan and the Japanese Biochemical Society, 2008 (BMB2008).



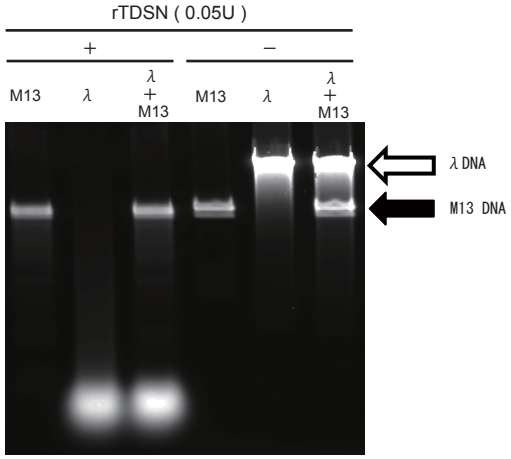
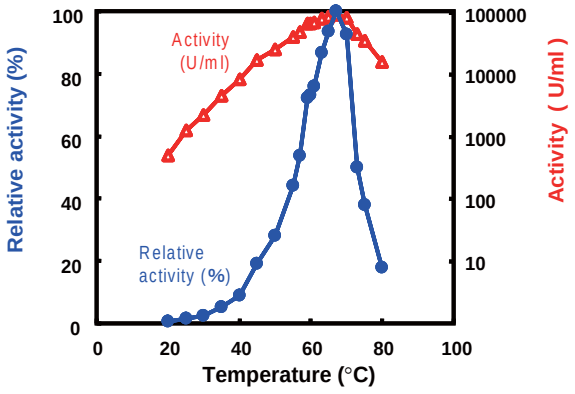


Figure 1. The rTDSN exhibit a strong preference for double double-stranded DNA substrate

In the case of the rTDSN-added reaction mixture (+) the double-stranded DNA substrate λ DNA (void arrow) alone was degraded, while the single-stranded DNA substrate M13 mp18 single strand DNA (black arrow) was not degraded.

On the other hand, when the dilution buffer was added instead of the enzyme (-) both of the substrate were not degraded.

The rTDSN hardly acts on the single-stranded DNA, it specifically degrades the double-stranded DNA alone.



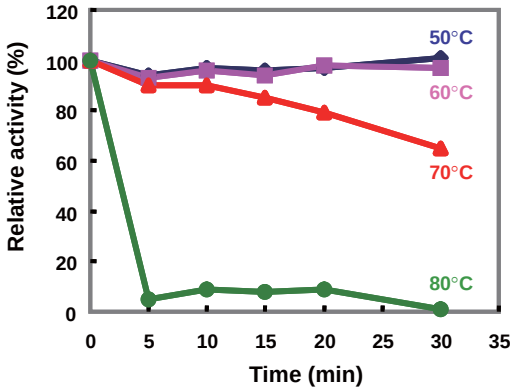


Figure 2. Temperature dependence of rTDSN activity.

DNase activities toward dsDNA were measured by modified Kunitz assay at various temperatures. Measured activity (U/ml) and relative activity (%) at each temperature were shown. The rTDSN showed high activity within the range of from about 20 ℃ to 70 ℃, its optimum activity temperature was about 67 ℃, and it showed an activity of 70% or more of the maximum activity at from about 60 ℃ to 70 ℃.

Figure 3. Kinetics of thermal inactivation of rTDSN.

After heat treatment of rTDSN at indicated temperatures, the relative activities of the rTDSN were determined. The DNase activities toward dsDNA were measured at 25℃. The rTDSN retained more than 90% activity after incubation at 50 ℃ or 60 ℃ for 30 minutes. The activity of the enzyme treated at 70 ℃ decreased to be about 60% after a lapse of time of 30 minutes. The activity of the enzyme treated at 80 ℃ disappeared after 5 minutes.

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