

Duplex-specific nuclease

- Specific to double-stranded DNA
- Thermostable
- Inhibited by EDTA

Description

Duplex-specific nuclease (DSN) is an enzyme purified from hepatopancreas of the Kamchatka crab (Shagin *et al.*, 2002). DSN shows a strong preference for cleaving double-stranded (ds) and DNA in DNA-RNA hybrid duplexes, compared with single-stranded (ss) DNA and RNA. Moreover, the cleavage rate of short, perfectly matched DNA duplexes by this enzyme is considerably higher than that for nonperfectly matched duplexes of the same length.

DSN find use in various applications to isolate single-stranded DNA from complex nucleic acids, for example in cDNA normalization method (Zhulidov *et al.*, 2004, 2005; Bogdanova *et al.*, 2007), for quantitative telomeric overhang determination (Zhao *et al.*, 2007), and for SNP detection (Shagin *et al.*, 2002).

Main properties of DSN

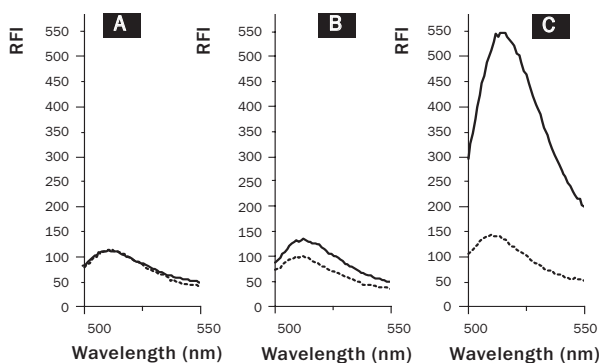
Substrate specificity

DSN exhibited strong cleavage preference for ds DNA substrates. No significant cleavage activity on RNA substrates and ss DNA is observed with working DSN concentrations (Zhao *et al.*, 2007). dsDNA:ssDNA cleavage ratio is about 1000. Moreover, the nuclease effectively cleaves DNA molecules in DNA-RNA hybrid duplexes.

Analysis of DSN action on synthetic oligonucleotide substrates revealed that the enzyme discriminates between perfectly matched short DNA-DNA duplexes (10-12 bp) and duplexes of the same length with at least one mismatch. It requires at least 10 bp DNA or 15 bp DNA-RNA perfect duplex for cleavage.

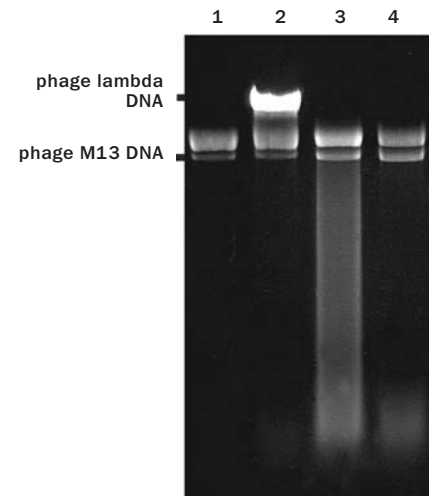
Biochemical properties

DSN acquires its enzymatic activity in the presence of divalent cations (Mn^{2+} , Co^{2+} , or Mg^{2+}). Mg^{2+} ion concentration for most applications should be at least 5 mM. DSN is inhibited by EDTA.



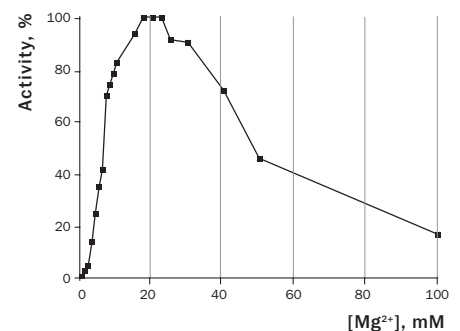
DSN action on one mismatch-containing (A, B) and perfectly matched (C) DNA duplexes.

Duplexes formed by 5-carboxyfluorescein (Fl)-5'-gccctatagt-3'-TAMRA signal probe and complementary targets (A — 5'-actcactataCggcgaat-3'; B — 5'-actcactataggTogaat-3'; C — 5'-actcactatagggcgaat-3') were incubated with DSN at 35°C for 15 min. Emission spectra were obtained on the spectrofluorimeter, with excitation at 480 nm. Dotted line - substrate fluorescence in the absence of enzyme; firm line - substrate fluorescence after incubation with DSN.



Action of DSN on ss DNA of phage M13 and ds DNA of phage lambda.

Lanes 1, 2 — negative controls, incubation without nuclease. 1 — phage M13 DNA alone, 2 — mixture containing phage M13 and lambda DNA. Lanes 3; 4 — digestion of phage M13 and lambda DNA mixture by DSN at 70°C for 1.5 min (lane 3) and 5 min (lane 4).



Effect of Mg-ions on DSN activity.

Activity of DNase on ds DNA substrate was measured using modified Kunitz assay (Kunitz, 1950) at different Mg^{2+} concentrations.

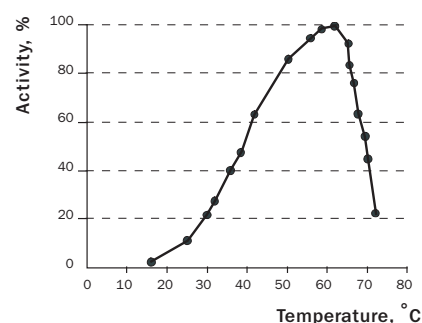
The temperature optimum for activity is 60°C. Despite a high optimal temperature, DSN retained only 10% activity as early as at 80°C. This sharp decrease in activity may be attributable, at least in part, to dsDNA substrate denaturation.

The optimal pH for DSN activity was estimated as 6.6. At pH values between 3 and 5, DSN displayed only 10% of its maximal activity. The nuclease is stable at a wide range of pH (from 4 to 12) and temperatures below 65°C. About 60% of DSN activity remains after 30-min incubation at 70°C, and 40% - after incubation at 80°C.

Incubation of DSN with aggressive chemicals like 1% SDS, 10 mM beta-mercaptoethanol, and 0.3% hydrogen peroxide at 37°C resulted in only a moderate drop in activity, and ~90% activity was maintained after 30 min incubation. However, a sharp decrease in activity was observed upon chemical treatment at 60°C. SDS completely inhibited DSN activity, while beta-mercaptoethanol and hydrogen peroxide induced approximately 70% and 80% loss in activity, respectively.

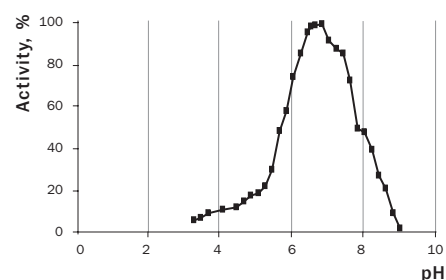
DSN is highly sensitive to ionic force (e.g., a 10 times decrease in catalytic activity is observed in the presence of 0.2 M NaCl). The addition of chaotropic agents or polyamines to the reaction mixture also resulted in suppression of enzyme activity.

DSN is tolerant to proteinase K treatment (incubation at 37°C for 30 min).



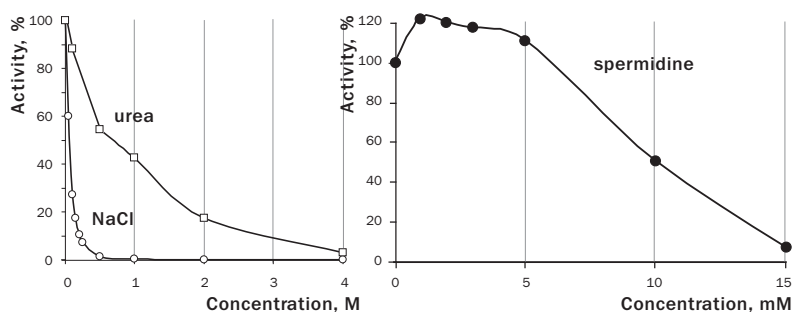
Dependence of the DSN activity from temperature.

Activity of DNase on ds DNA substrate was measured using modified Kunitz assay (Kunitz, 1950) at different temperature.



Effect of pH on DSN activity.

Activity of DNase on ds DNA substrate was measured using modified Kunitz assay (Kunitz, 1950) at different pH.



Inhibition of DSN by ionic force, urea, and spermidine.

100% represents DSN activity at zero concentration of the agent.

DSN licensing opportunities

Evrogen technology embodied in DSN is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market-driven license options are offered for upgrade and novel development of products and applications.

For licensing information, please contact Evrogen at license@evrogen.com.

References

- Bogdanova E. *et al.* (2007) Molecular BioSystems, in press.
- Kunitz M. (1950) J Gen Physiol, 33, 363-377.
- Shagin D. *et al.* (2002) Genome Res. 12, 1935-1942.
- Zhulidov P. *et al.* (2004) Nucleic Acid Res., 32: e37.
- Zhulidov P. *et al.* Russian Journal of Bioorganic Chemistry, 31: 170 - 177.
- Zhao Y. *et al.* (2007) Nucleic Acid Res., doi:10.1093/nar/gkm1063.

Available DSN compositions

Product	Cat.#	Description	Size
Duplex-specific nuclease (lyophilized)	EA001	Each package comprises a detailed instruction,	50 Units
	EA002	lyophilized DSN, DSN storage and working buffers,	100 Units
	EA003	and control DNA sample	10 Units

Please contact your local distributor for exact prices and delivery information.

DSN was purified from Kamchatka crab hepatopancreas using modified protocol from (Shagin *et al.*, 2002). Lyophilized DSN enzyme must be stored at +4°C before resolution. Other components should be stored at -20°C. DNAase activity was measured using modified Kunitz assay (Kunitz, 1950), where unit definition was defined as: the amount of DSN added to 50 µg/ml calf thymus DNA that causes an increase of 0.001 absorbance units per minute. Activity assay was performed at 25°C, in 50 mM Tris-HCl buffer, pH 7.15, containing 5 mM MgCl₂.

Other DSN-related products

Product	Cat.#	Description	Size
Trimmer kit	NK001	cDNA normalization kit allowing normalization of full-length-enriched cDNA for nondirectional cloning	10 rxn
Trimmer-Direct kit	NK002	cDNA normalization kit allowing normalization of full-length-enriched cDNA for directional cloning	10 rxn

Please contact your local distributor for exact prices and delivery information.

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MATERIAL SAFETY DATA SHEET INFORMATION

To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.