evrogen

Duplex-specific nuclease

- Thermostable nuclease, specific to double-stranded DNA

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), for quantitative telomeric overhang determination (Zhao et al., 2007), and for SNP detection (Shagin *et al.*, 2002).

Product	Cat.#	Size
Duplex-specific	EA001	50 Units
nuclease	EA002	100 Units
(lyophilized)	EA003	10 Units

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 $MgCl_2$.

Reagents and storage conditions

Reagent	Composition	Amount
DSN storage buffer	50 mM Tris-HCl, pH 8.0	100 µl
10X DSN master buffer	500 mM Tris-HCl, pH 8.0; 50 mM $MgCl_2$; 10 mM DTT	100 µl
2X DSN stop solution	10 mM EDTA	500 µl
Control template	plasmid DNA, 100ng/µl	20 µl

Reagents required but not included:

Glycerol, 100%; sterile water; agarose gel electrophoresis reagents.

Storage:

Lyophilized duplex-specific nuclease (DSN) must be stored at +4 °C. After dilution in the DSN storage buffer as described in the section "DSN dilution", DSN solution must be stored at -20°C. Other components must be stored at -20°C.

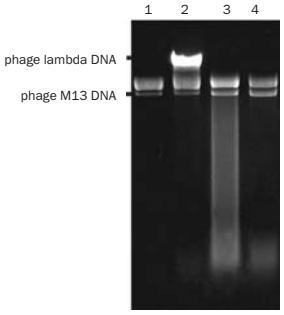
DSN substrate specificity

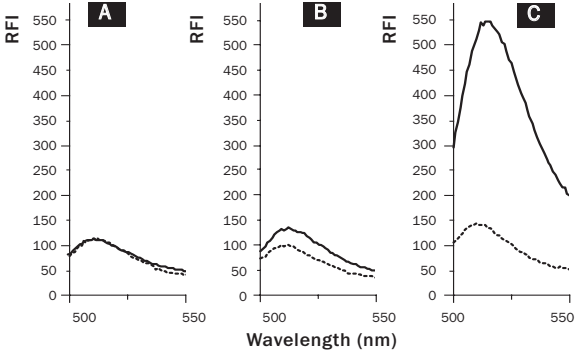
DSN exhibited strong cleavage preference for ds DNA substrates. No significant cleavage activity on RNA substrates and ss DNA is observed with working DSN concentrations (Zhao et al., 2007). dsDNA:ssDNA cleavage ratio is about 1000. The nuclease effectively cleaves DNA molecules in DNA-RNA hybrid duplexes.

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.

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).





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

Duplexes formed by 5-carboxyfluorescein (FI)-5'-gccctatagt-3'-TAMRA signal probe and complementary targets (A — 5'-actcactataCggcgaat-3'; B — 5'-actcactataggTcgaat-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.

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.

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 a moderate drop in activity, and ~90% activity was maintained after 30 min incubation. However, upon 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.

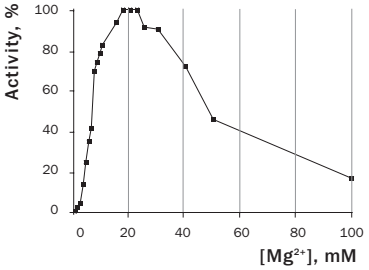
DSN is tolerant to proteinase K treatment (incubation at 37°C for 30 min).

Purity

DSN was purified from Kamchatka crab hepatopancreas using modified procedure described in (Shagin *et al*., 2002).

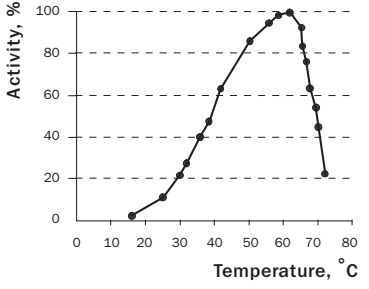
Effect of Mg-ions on the DSN activity.

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



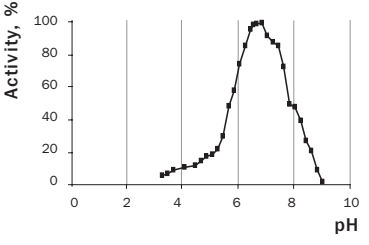
Dependence of the DSN activity from temperature.

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



Effect of pH on the DSN activity.

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



DSN dilution protocol

Dilute the lyophilized DSN enzyme as follows:

1. Add DSN storage buffer to the lyophilized DSN enzyme on the basis of 5 µl of the buffer for each 10 DSN units.

2. Mix contents by gently flicking the tube. Spin tube briefly in a microcentrifuge. Avoid foaming of the mixture.

3. Incubate the tube at room temperature for 5 min.

4. Add equal amount of 100% glycerol (to 50% final glycerol concentration) to the tube. Mix contents by gently flicking the tube. Spin the tube briefly in a microcentrifuge. Avoid foaming of the mixture.

5. Store the DSN solution at -20°C.

DSN activity testing

Important note: We strongly recommend performing this procedure before enzyme use.

1. Combine the following reagents in the order shown:

12 µl	Sterile water (not included)
4 µl	DSN control template
2 µl	10XDSN master buffer
18 µl	**Total Volume**

2. Mix contents and spin the tube briefly in a microcentrifuge.

3. Aliquot 9 µl of the reaction mixture into each of the two sterile PCR tubes labeled C (control) and E (experimental).

4. Add 1 µl of DSN storage buffer into C-tube. Mix contents and spin the tube briefly in a microcentrifuge.

5. Add 1 µl of DSN solution into the E-tube. Mix contents by gently flicking the tube. Spin the tube briefly in a microcentrifuge.

6. Overlay the reaction mixture in each tube with drop of mineral oil and spin the tubes briefly in a microcentrifuge.

7. Incubate the tubes in a thermal cycler at 65°C for 10 min.

8. To inactivate DSN enzyme, add 5 µl of 2X DSN stop solution, mix contents and spin the tube briefly in a microcentrifuge. Place the tubes at room temperature.

9. Electrophorese 5 µl of each reaction mixture alongside 0.1 µg of 1-kb DNA size markers on a 1.5% agarose/EtBr gel in 1X TAE buffer. Using electrophoresis data, estimate condition of your DSN enzyme. For comparison, Figure at the page 8 shows the typical gel profile of "DSN control template" digested by DSN with successful activity and by partially inactive DSN.

DSN treatment: reaction conditions

1. Combine the following reagents in the order shown:

Y µl	50 - 500 ng DNA (in sterile water)
X µl	Sterile water
1 µl	10X DSN master buffer
1 µl	DSN solution
10 µl	**Total Volume**

2. Mix contents gently and spin the tube briefly in a microcentrifuge.

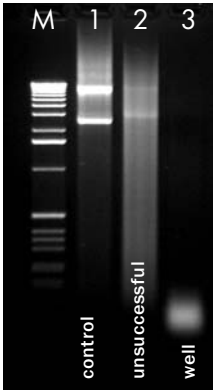
3. If necessary, overlay the reaction mixture with a drop of mineral oil and spin the tube briefly in a microcentrifuge.

4. Incubate the tube in a thermal cycler at 65°C for 7-20 min.

Note: Incubation time depends on the complexity of the sample and particular tasks. Depending on your particular needs, incubation temperature can be changed from 35 to 70°C; however, incubation time/DSN concentration in the reaction mixture should be optimized additionally.

5. To inactivate DSN enzyme, add 5 µl of 2X DSN stop solution, mix contents and spin the tube briefly in a microcentrifuge.

6. Incubate the tube in a thermal cycler at reaction temperature for 5 min.



DSN activity testing.

Samples containing 200 ng of plasmid DNA were incubated with or without DSN in 1x DSN master buffer for 7 min at 65°C. Reactions were stopped by EDTA and digestion products were electrophoresed on a 1.5% agarose/EtBr gel in 1X TAE buffer.
Lane 1 - control DNA (incubation without DSN).
Lane 2 - DNA incubated with unsuccsessful DSN enzyme.
Lane 3 - successful digestion of DNA by DSN.
Lane M - 1 kb DNA size marker.

References

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.

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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.