

## Duplex-specific nuclease

Duplex-specific nuclease (DSN) is an enzyme purified from hepatopancreas of Red King (Kamchatka) crab [1]. DSN shows a strong preference for cleaving double-stranded (ds) DNA and DNA in DNA-RNA hybrid duplexes, and is practically inactive towards single-stranded (ss) DNA or single or double-stranded RNA. Moreover, the enzyme is capable to discriminate between perfectly and nonperfectly matched short DNA duplexes. DSN is widely used for full-length enriched cDNA normalization [2, 3] and for construction of normalized RNA-seq libraries for next generation sequencing ([4], Illumina DSN normalization protocol). The list of approved DSN applications also includes normalization of genomic DNA [5], cDNA depletion and ribosomal cDNA depletion [6, 7, 8], cDNA subtraction [9], SNP detection [1, 10], construction of repeat-free FISH probes [11], multiplexed fluorescence detection of miRNAs [12], quantitative telomeric overhang determination [13, 14], etc.

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

DNAase activity was measured using modified Kunitz assay [15], where unit was defined as: the amount of DSN added to 50  $\mu\text{g}/\text{ml}$  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  $\text{MgCl}_2$ .

### List of kit components:

Component	Composition	Amount
DSN enzyme	lyophilized	10/50/100 units
DSN storage buffer	50 mM Tris-HCl, pH 8.0	120 $\mu\text{l}$
10X DSN master buffer	500 mM Tris-HCl, pH 8.0; 50 mM $\text{MgCl}_2$ ; 10 mM DTT	200 $\mu\text{l}$
2X DSN stop solution	10 mM EDTA	1000 $\mu\text{l}$
Control template	plasmid DNA, 100ng/ $\mu\text{l}$	20 $\mu\text{l}$

**Reagents required but not included:** Glycerol, 100%; sterile water; agarose gel electrophoresis reagents.

**Shipping and Storage conditions:** All components of the kit can be shipped at ambient temperature. Upon arrival all components must be stored at -20°C.

### Notice to Purchaser:

The Product is intended for research use only.

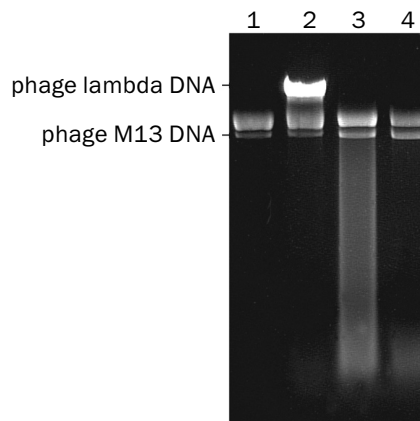
DSN-related products are covered by U.S. Patents No. 7,435,794 and 7,803,922. By use of these products, you accept the terms and conditions of the applicable Limited Use Label License #002: <http://www.evrogen.com/products/Evrogen-NA-license.shtml>.

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## DSN substrate specificity

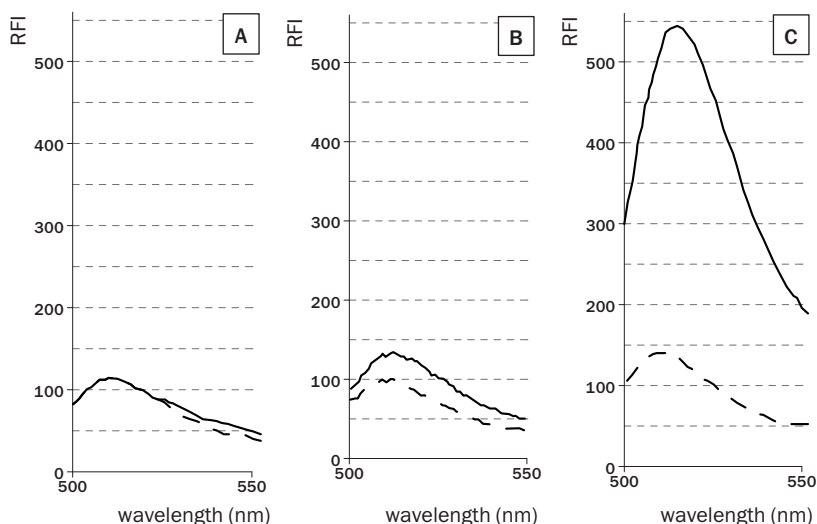
DSN exhibits strong cleavage preference for ds DNA substrates. It can show minor activity against ss DNA when both DSN enzyme and substrate are present at high concentrations, however this activity is not detectable in presence of competitive ds DNA and RNA [13]. DSN does not cleave RNA, but effectively cleaves DNA in DNA-RNA hybrid duplexes [1].

Analysis of DSN activity 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 (3) and 5 min (4).



**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'-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

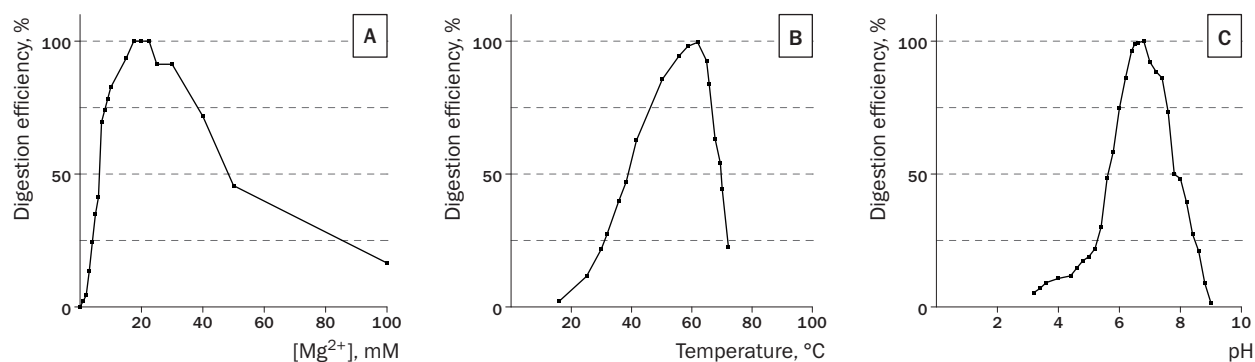
DSN acquires its enzymatic activity in the presence of divalent cations ( $Mn^{2+}$ ,  $Co^{2+}$ , or  $Mg^{2+}$ ).  $Mg^{2+}$  ion concentration for most applications should be at least 5 mM. DSN is inhibited by EDTA.

The temperature optimum for DSN activity is 60°C. However, already at 80°C DSN retains only 10% of activity. This sharp decrease in activity may be attributable, at least in part, to ds DNA substrate denaturation. The optimal pH for DSN activity is 6.6. At pH values between 3 and 5, DSN displays 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 results in only a moderate drop in activity, after 30 min incubation about 90% of activity is preserved. However, a sharp decrease in activity was observed upon chemical treatment at 60°C. SDS completely inhibits DSN activity, while  $\beta$ -mercaptoethanol and hydrogen peroxide induce 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 results in suppression of enzyme activity. DSN is tolerant to proteinase K treatment (incubation at 37°C for 30 min).



**A. Effect of Mg<sup>2+</sup> ions on the DSN activity.** Activity of DNase on ds DNA substrate was measured using modified Kunitz assay [15] at different Mg<sup>2+</sup> concentrations.

**B. Dependence of the DSN activity from temperature.** Activity of DNase on ds DNA substrate was measured using modified Kunitz assay [15] at different temperature.

**C. Effect of pH on the DSN activity.** Activity of DNase on ds DNA substrate was measured using modified Kunitz assay [15] 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  $\mu$ l 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.

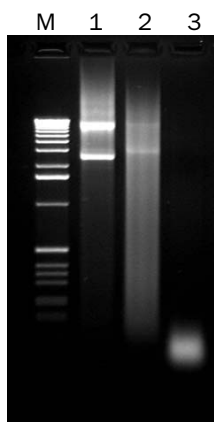
## DSN activity testing

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

1. Combine the following reagents in the order shown:
 

12 $\mu$ l	Sterile water (not included)
4 $\mu$ l	DSN control template
2 $\mu$ l	10XDSN master buffer
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18 $\mu$ l	Total Volume
2. Mix contents and spin the tube briefly in a microcentrifuge.
3. Aliquot 9  $\mu$ l of the reaction mixture into each of the two sterile PCR tubes labeled C (control) and E (experimental).
4. Add 1  $\mu$ l of DSN storage buffer into C-tube. Mix contents and spin the tube briefly in a microcentrifuge.
5. Add 1  $\mu$ l 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.

- To inactivate DSN enzyme, add 5  $\mu\text{l}$  of 2X DSN stop solution, mix contents and spin the tube briefly in a microcentrifuge. Place the tubes at room temperature.
- Load 5  $\mu\text{l}$  of each reaction mixture alongside 0.1  $\mu\text{g}$  of 1-kb DNA size markers onto a 1.5% agarose/EtBr gel in 1X TAE buffer. Using electrophoresis data, estimate condition of your DSN enzyme. For comparison, refer to the figure below.



#### 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. Lane 1 - control DNA (incubation without DSN). Lane 2 - DNA incubated with partially inactive DSN. Lane 3 - successful digestion of DNA by DSN. Lane M - 1 kb DNA size marker.

### DSN treatment: reaction conditions

- Combine the following reagents in the order shown:

Y $\mu\text{l}$	50 - 500 ng DNA (in sterile water)
X $\mu\text{l}$	Sterile water
1 $\mu\text{l}$	10X DSN master buffer
1 $\mu\text{l}$	DSN solution
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10 $\mu\text{l}$	Total Volume

- Mix contents gently and spin the tube briefly in a microcentrifuge.
- If necessary, overlay the reaction mixture with a drop of mineral oil and spin the tube briefly in a microcentrifuge.
- 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 varied from 35 to 70°C; in this case incubation time and DSN concentration should be optimized additionally.
- To inactivate DSN enzyme, add 5  $\mu\text{l}$  of 2X DSN stop solution, mix contents and spin the tube briefly in a microcentrifuge.
- Incubate the tube in a thermal cycler at reaction temperature for 5 min.

### References

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