

## Product Information

# Deoxyribonuclease I from bovine pancreas

Sigma Type II-S, lyophilized powder, protein  $\geq 80\%$ ,  $\geq 2000$  units/mg protein**D4513**

## Product Description

CAS Registry Number: 9003-98-9

Enzyme Commission (EC) Number: 3.1.21.1

Synonyms: DNase I, Deoxyribonuclease A, Deoxyribonuclease 5'-oligonucleotidohydrolase

Deoxyribonuclease I (DNase I) is an endonuclease that cleaves DNA by preferentially acting on phosphodiester bonds adjacent to pyrimidines, to produce polynucleotides with terminal 5'-phosphates. A tetranucleotide is the smallest average digestion product. In the presence of  $Mg^{2+}$  ions, DNase I attacks each strand of DNA independently and the cleavage sites are random. If  $Mn^{2+}$  ions are present, both DNA strands are cleaved at approximately the same site.<sup>1</sup> DNase I hydrolyzes single-stranded DNA, double-stranded DNA, and chromatin (the reaction rate is restricted by DNA association with histones).

DNase I is found in most cells and tissues. In mammals, the pancreas is one of the best sources for the enzyme. Pancreatic DNase I was the first DNase to be isolated. The calculated molecular mass is 30,072 Da. DNase I exists as a mixture of glycoproteins with two disulfide bridges.<sup>2</sup>

Bovine pancreatic DNase I contains four chromatographically distinguishable components, labeled A, B, C, and D.<sup>3</sup> The molar ratios of A:B:C in a pancreatic extract are 4:1:1. Only minor amounts of D are found. Forms A and B differ in carbohydrate content (see Table below).<sup>4</sup>

### Carbohydrate Content<sup>4</sup>

Carbohydrate / Form	A	B	C
<i>N</i> -Acetylglucosamine	2	3	2
Mannose	6	5	5
Sialic Acid	-	1	-
Galactose	-	1	-

Form C differs from Forms A and B by having one less His and one more Pro, and in the carbohydrate chain.<sup>4</sup>

DNase I is used to remove DNA from protein and nucleic acid samples, and to nick DNA as a first step to incorporate labeled bases into DNA. Several theses<sup>5-10</sup> and dissertations<sup>11-26</sup> have cited use of product D4513 in their protocols.

Isoelectric points:<sup>2</sup>

- A: 5.22
- B: 4.96
- C: 5.06
- D: 4.78

Optimal pH: 7-8

Extinction Coefficient:  $E_{280}^{1\%} = 11.1$ 

### Activators

- DNase I has an absolute requirement for divalent metal cations.
- The most commonly used divalent metal cation is  $Mg^{2+}$ .<sup>27,28</sup>
- However,  $Mn^{2+}$ ,  $Ca^{2+}$ ,  $Co^{2+}$ , and  $Zn^{2+}$  will activate DNase I.<sup>27-29</sup>
- 5 mM  $Ca^{+2}$  will stabilize DNase I against proteolytic digestion.<sup>30</sup>
- 0.1 mM  $Ca^{+2}$  is needed to reduce the rate of inactivation by one-half.<sup>30</sup>

### Inhibitors

There is no general inhibitor specific for DNase I.<sup>27,28</sup> Citrate inhibits  $Mg^{2+}$ -activated DNase I, but not  $Mn^{2+}$ -activated DNase I.

- 2-Mercaptoethanol (the reduced enzyme is inactive, but can be reactivated in the presence of  $Ca^{2+}$  or  $Mg^{2+}$  ions)<sup>28</sup>
- Chelators (such as EDTA, EGTA)
- Sodium dodecyl sulfate (SDS)<sup>30</sup>
- Actin<sup>32</sup>

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Product

This product is chromatographically purified from bovine pancreas. The purification procedure is not selective for any form (A, B, C, or D) of DNase I. D4513 is supplied as a lyophilized powder, containing CaCl<sub>2</sub>. This product is 0.2 µm sterile-filtered. Each vial contains ~11 mg of protein.

Protein content: ≥ 80% (Biuret)

Specific activity: ≥ 2000 Kunitz units/mg protein

Unit Definition:<sup>33</sup>

- One Kunitz unit will produce a  $\Delta A_{260}$  of 0.001 per minute per mL at pH 5.0 at 25 °C, using DNA, Type I or III, as substrate, with  $[Mg^{2+}] = 4.2$  mM.
- This enzyme assay reaction is performed in 95 mM acetate buffer, pH 5.0, at 25 °C, containing 4.75 mM Mg<sup>2+</sup> and 1.9 mM Ca<sup>2+</sup>, in a 3 mL reaction.

Contaminants:

- Protease: ≤ 0.005%
- RNase: ≤ 0.01%
- Chymotrypsin: ≤ 0.01%
- Endotoxin-tested

## Preparation Instructions

This enzyme is soluble in 0.15 M NaCl at 5 mg/mL.

## Storage/Stability

DNase I retains activity for at least three years, when unopened and stored long-term at the recommended temperature, -20 °C.

Solutions of DNase I at 10 mg/mL in 0.15 M NaCl may lose <10% of its activity when stored for a week in aliquots at -20 °C. The same solutions stored in aliquots at 2-8 °C can lose ~20% activity.

DNase I remains active in solution between pH 5-7 up to 60 °C for at least five hours. A 1 mg/mL solution in acetate buffer (pH 5.0) or Tris buffer (pH 7.2) loses activity at the rate of 6% per hour. At 68 °C, DNase I loses activity in <10 minutes.

## References

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