

## Product Information

### Deoxyribonuclease I from bovine pancreas

Product Number **D4263**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

CAS RN 9003-98-9  
EC 3.1.21.1  
Synonyms: DNase I; Deoxyribonuclease  
5'-Oligonucleotidohydrolase

#### Product Description

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  $\text{Mg}^{2+}$  ions, DNase I attacks each strand of DNA independently and the cleavage sites are random. If  $\text{Mn}^{2+}$  ions are present, both DNA strands are cleaved at approximately the same site.<sup>1</sup> DNase I hydrolyzes single and double-stranded DNA and chromatin (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 isolated. The calculated molecular mass is 30,072 Da. DNase I exists as a mixture of glycoproteins with two disulfide bridges.<sup>2</sup>

#### Carbohydrate Content:<sup>3</sup>

| Form:               | <u>A</u> | <u>B</u> | <u>C</u> |
|---------------------|----------|----------|----------|
| N-Acetylglucosamine | 2        | 3        | 2        |
| Mannose             | 6        | 5        | 5        |
| Sialic Acid         | —        | 1        | —        |
| Galactose           | —        | 1        | —        |

Bovine pancreatic DNase I contains four chromatographically distinguishable components, 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. Form C differs from forms A and B by having one less histidine and one more proline, 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.

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 is  $\text{Mg}^{2+}$ .<sup>5</sup> However,  $\text{Mn}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Zn}^{2+}$  will activate DNase I.<sup>5,6</sup> A concentration of 5 mM  $\text{Ca}^{+2}$  will stabilize DNase I against proteolytic digestion; 0.1 mM is needed to reduce the rate of inactivation by one-half.<sup>7</sup>

#### Inhibitors:

2-Mercaptoethanol (the reduced enzyme is inactive, but can be reactivated in the presence of  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  ions);<sup>6</sup> chelators; sodium dodecyl sulfate (SDS);<sup>8</sup> and actin.<sup>9</sup> There is no general inhibitor specific for DNase I.<sup>5</sup> Citrate inhibits  $\text{Mg}^{2+}$ -activated DNase I, but not  $\text{Mn}^{2+}$ -activated DNase I.

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. It is supplied as a lyophilized powder, containing  $\text{CaCl}_2$ .

Vial content: ~2,000 Kunitz units/vial  
 $\geq 0.25$  mg protein (biuret)/vial

Unit definition: One Kunitz unit will produce a change in  $A_{260}$  of 0.001 per minute per ml at pH 5.0 at  $25\text{ }^{\circ}\text{C}$  using DNA, Type I or III, as the substrate. This enzyme assay reaction is performed in 95 mM acetate buffer, pH 5.0, at  $25\text{ }^{\circ}\text{C}$ , containing 4.75 mM  $\text{Mg}^{2+}$  and 1.9 mM  $\text{Ca}^{2+}$ , in a 3 ml reaction.

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

This enzyme is soluble in 0.15 M NaCl (5 mg/ml), yielding a clear solution.

### Storage/Stability

DNase I retains activity for at least three years when stored at -20 °C.

Solutions of DNase I (10 mg/ml) in 0.15 M NaCl may lose <10% of its activity 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 and 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%/hour. At 68 °C, DNase I loses activity in <10 minutes.

### References

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VLR,JWM,RBG,AI,GCY,MAM 01/17-1