

## Product Information

# Deoxyribonuclease I from bovine pancreas

Sigma Type IV, lyophilized powder,  $\geq 2000$  Kunitz units/mg protein**D5025**

## 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
N-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>5-7</sup> and dissertations<sup>8-26</sup> have cited use of product D5025 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 purified from bovine pancreas. The purification procedure is not selective for any form (A, B, C, or D) of DNase I. D5025 is supplied as a lyophilized powder, containing  $\text{CaCl}_2$ .

Protein content:  $\geq 80\%$

Specific activity:  $\geq 2000$  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  $[\text{Mg}^{2+}] = 4.2$  mM.
- This enzyme assay reaction is performed in 95 mM acetate buffer, pH 5.0, at 25 °C, containing 4.75 mM  $\text{Mg}^{2+}$  and 1.9 mM  $\text{Ca}^{2+}$ , in a 3 mL reaction.

Contaminants:

- Protease:  $\leq 0.05\%$
- RNase:  $\leq 0.02\%$
- Chymotrypsin:  $\leq 0.5\%$

## Preparation Instructions

This product is tested for solubility in 0.15 M NaCl at 5 mg/mL. One publication has reported preparation of stock solutions of this DNase I product at 20 mg/mL in Ringer's solution,<sup>34</sup> although we have not tested this condition ourselves.

## Storage/Stability

D5025 has a recommended retest date of 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  $\sim 20\%$  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.

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