

Product Information

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Deoxyribonuclease I from bovine pancreas

Catalog Number **D5025**
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

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.

DNase I is an endonuclease that acts 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.¹ DNase I hydrolyzes single and double-stranded DNA and chromatin (reaction rate is restricted by DNA association with histones).

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.

Bovine pancreatic DNase consists four chromatographically distinguishable components, A, B, C, and D.² 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.⁴

Molecular mass: 30,072 Da (peptide, calculated), exists as a mixture of glycoproteins with two disulfide bridges.³

Carbohydrate Content:²

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

Isoelectric points:² 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 Mg^{2+} ;⁵ however, Mn^{2+} , Ca^{2+} , Co^{2+} , and Zn^{2+} will activate DNase I.^{5,6} A concentration of 5 mM Ca^{+2} will stabilize DNase I against proteolytic digestion; 0.1 mM is needed to reduce the rate of inactivation by one-half.⁷

Inhibitors:

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

This product is chromatographically purified from New Zealand source 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 CaCl_2 .

Specific activity: ≥ 2000 Kunitz units/mg protein

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 83 mM acetate buffer, pH 5.0, at $25\text{ }^{\circ}\text{C}$, containing 4.2 mM Mg^{2+} , in a 3 ml reaction.

Protein: $\geq 80\%$ (biuret)

Contaminants:

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

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.

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