Product Information

Deoxyribonuclease I from bovine pancreas

Sigma Type II, lyophilized powder, protein ≥80%, ≥2000 units/mg protein

D4527

Product Description

CAS Registry Number: 9003-98-9

Enzyme Commission (EC) Number: 3.1.21.1

Synonyms: DNase I, Deoxyribonuclease A, Deoxyribonucleate 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.[1] 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.[2]

Bovine pancreatic DNase I contains four chromatographically distinguishable components, labeled A, B, C, and D.[3] 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).[4]

Carbohydrate Content[4]

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.[4]

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[5-8] and dissertations[9-27] have cited use of product D4527 in their protocols.

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 divalent metal cation is Mg^{2+}.[27,28]
- However, Mn^{2+}, Ca^{2+}, Co^{2+}, and Zn^{2+} will activate DNase I.[27-29]
- 5 mM Ca^{+2} will stabilize DNase I against proteolytic digestion.[30]
- 0.1 mM Ca^{+2} is needed to reduce the rate of inactivation by one-half.[30]

Inhibitors

There is no general inhibitor specific for DNase I.[27,28] 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)[28]
- Chelators (such as EDTA, EGTA)
- Sodium dodecyl sulfate (SDS)[30]
- Actin[32]

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. D4527 is supplied as a lyophilized powder, containing $CaCl_2$.

Protein content: ≥ 80% (Biuret)

Specific activity: ≥ 2000 Kunitz units/mg protein

Unit Definition:[33]

- One Kunitz unit will produce a ΔA_{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^{2+} and 1.9 mM Ca^{2+}, in a 3 mL reaction.

Contaminants:

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

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.

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