Product Information

Deoxyribonuclease I from bovine pancreas

Lyophilized powder, protein ≥85%, ≥400 Kunitz units/mg protein

DN25

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] and dissertations[6-24] have cited use of product DN25 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+}.[25,26]
- However, Mn^{2+}, Ca^{2+}, Co^{2+}, and Zn^{2+} will activate DNase I.[125-27]
- 5 mM Ca^{+2} will stabilize DNase I against proteolytic digestion.[28]
- 0.1 mM Ca^{+2} is needed to reduce the rate of inactivation by one-half.[28]

Inhibitors

There is no general inhibitor specific for DNase I.[25,26] 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)[27]
- Chelators (such as EDTA, EGTA)
- Sodium dodecyl sulfate (SDS);[29] and actin[30]

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

Specific activity: ≥ 400 Kunitz units/mg protein

Unit Definition:[31]

- 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.

Storage/Stability

DN25 has a recommended retest date of four years, when unopened and stored long-term at the recommended temperature, –20 °C.

Preparation Instructions

DNase I is soluble in 0.15 M NaCl at 5 mg/mL. 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.

Alternatively, solutions at a minimum concentration of 1 mg protein/mL, with 5 mM $CaCl_2$ and stored at –20 °C, may retain >90% activity for at least a year. Solutions containing <0.1 mg protein/mL are considerably less stable, and may require gelatin as stabilizer.[32]

For applications unaffected by glycerol, two other storage buffers are options, as these formulations do not freeze at –20 °C:

- 20 mM sodium acetate (pH 6.5), containing 5 mM $CaCl_2$ and 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 50% (v/v) glycerol, with DNase I at ≤5 mg/mL
- 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 50% (v/v) glycerol, with DNase I at ≤2 mg/mL[5]

References

1. Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd edition. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY), Vol. 2, p. 5.83 (1989).
2. Liao, T.-H. *et al.*, *J. Biol. Chem.*, **248(4)**, 1489-1495 (1973).
3. Kim, H.S., and Liao, T.-H., *Anal. Biochem.*, **119(1)**, 96-101 (1982).
4. Salnikow, J. *et al.*, *J. Biol. Chem.*, **245(21)**, 5685-5690 (1970).
5. Berthelet, Sharon, "Integrated Analysis of the Multifunctional Protein Aft1p". University of Ottawa, M.Sc., thesis, p. 64 (2008).
6. Rodriguez, Tristan Argéo, "An Investigation into a Meiotic Checkpoint that Monitors Sex Chromosome Pairing". University College London, Ph.D. dissertation, p. 226 (1997).
7. Abbadi, Amineh M., "Hyaluronan Rafts on Airway Epithelial Cells". Cleveland State University, Ph.D. dissertation, p. 20 (2008).
8. Miller, Matthias, "Towards the generation of novel natural killer cell-based immunotherapies against melanoma". Ruperto-Carola University Heidelberg, Dr. rer. nat. dissertation, p. 46 (2014).
9. Dembla, Mayur Harish, "ArfGAP3 Is a Component of the Photoreceptor Synaptic Ribbon Complex and Forms an NAD(H)-Regulated, Redox-Sensitive Complex with RIBEYE That Is Important for Endocytosis". Saarland University, Ph.D. dissertation, pp. 43, 65 (2015).
10. Fütterer, Jane Wong, "Characterisation of a novel protein, ANKRD18A, implicated in a severe form of thrombocytopenia". University of Birmingham, Ph.D. dissertation, p. 105 (2016).
11. Kong, Linghai, "Regulation of System Xc-by the Neuropeptide PACAP: Implications for Glutamate Transmission in Drug Addiction". Marquette University, Ph.D. dissertation, p. 86 (2017).
12. Pennington, Shaun Harry, "The human immune response to oral vaccination with live-attenuated Salmonella Typhi (Ty21a)". University of Liverpool, Ph.D. dissertation, p. 50 (2017).
13. Siller, Saul Sylvan, "The Centriolar Distal Appendage Proteins CEP164 and Chibby1 Coordinate Multiple Steps in the Formation and Maintenance of Airway Multicilia". Stony Brook University, Ph.D. dissertation, p. 39 (2017).
14. Dell'Aringa, Mark, "The Role of Notch Signaling in Type-2 Immunity". Duke University, Ph.D. dissertation, pp. 78, 80 (2018).

15. Emrick, Joshua James, "Deficiency in a putative glutathione transferase produces atopic dermatitis with mast cell-mediated histaminergic itch". University of California San Francisco, Ph.D. dissertation, p. 25 (2018).
16. Klukovich, Rachel A., "Epigenetic regulation of spermatogenesis and transgenerational epigenetic inheritance". University of Nevada, Reno, Ph.D. dissertation, pp. 120, 152 (2018).
17. Maurer, Deena Marie, "Dysregulated NF-κB – dependent ICOSL Expression in Human Dendritic Cell Vaccines Impairs T Cell Responses in Melanoma Patients". University of Pittsburgh School of Medicine, Ph.D. dissertation, p. 36 (2020).
18. Munson, Paul Veness, "Therapeutic conserved elements (CE) DNA vaccines in simian-human immunodeficiency virus (SHIV) or SIV-infected macaques". University of Washington, Ph.D. dissertation, p. 67 (2018).
19. Poon, James, "Tissue Engineering Architectural Cues for *in vitro* Models of Respiratory Epithelium". University of Toronto, Ph.D. dissertation, p. 79 (2018).
20. Hähle, Andreas, "Elucidation of the molecular and cellular mechanisms of FKBP51-selective inhibitors". Technische Universität Darmstadt, Dr. rer. nat. dissertation, p. 19 (2019).
21. Hesterberg, Rebecca Swearingen, "Understanding immunometabolic and suppressive factors that impact cancer development and progression". University of South Florida, Ph.D. dissertation, p. 58 (2019).
22. O'Neal, Troy Justin, "The early involvement of CNS-draining lymphatics and host response to West Nile virus infection". Vanderbilt University, Ph.D. dissertation, pp. 23, 41 (2020).
23. Shiuan, Eileen Fong, "The Epha2/Ephrin-A1 Axis Regulates Host-Tumor Interactions". Emory University, Ph.D. dissertation, p. 76 (2020).
24. Lassi, Maximillian, "Paternal Circadian Arrhythmia Affects Offspring Health and Feeding Behavior". Technische Universität München, Dr. rer. nat. dissertation, p. 45 (2021).
25. Weir, A.F., *Methods Mol. Biol.*, **16**, 1-6 (1993).
26. Weir, A.F., *Methods Mol. Biol.*, **16**, 7-16 (1993).
27. Price, P.A. *et al.*, *J. Biol. Chem.*, **244(4)**, 929-932 (1969).
28. Price, P.A. *et al.*, *J. Biol. Chem.*, **244(4)**, 917-923 (1969).
29. Liao, T.-H., *J. Biol. Chem.*, **250(10)**, 3831-3836 (1975).
30. Lazarides, E., and Lindberg, U., *Proc. Natl. Acad. Sci. USA*, **71(12)**, 4742-4746 (1974).
31. Kunitz, M., *J. Gen. Physiol.*, **33(4)**, 349-362 (1950).
32. McDonald, M.R., *Methods Enzymol.*, **2**, 437-447 (1955).

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