Catalog Number: 100574, 100575, 190062

Deoxyribonuclease I

CAS #: 9003-98-9

Description: Deoxyribonuclease from beef pancreas, DNase I, was first crystallized by Kunitz.[10,11] It is an endonuclease which splits phosphodiester linkages, preferentially adjacent to a pyrimidine nucleotide yielding 5'-phosphate terminated polynucleotides with a free hydroxyl group on position 3'. The average chain of limit digest is a tetranucleotide.[19] DNase I acts upon single chain DNA[29], and upon double-stranded DNA and chromatin. In the latter case, although histones restrict susceptibility to nuclease action, over a period of time nearly all chromatin DNA is acted upon. According to Mirsky and Silverman[20], this could result from the looseness of histone attachment to DNA. They found that lysine-rich histones more effectively block DNase access to DNA than arginine-rich histones. Billing and Bonner[2] suggest that DNase attacks the histone-free strand of chromatin DNA. Schmidt, et. al.[28] indicate that hydrolysis of the histone-free region of DNA strands accounts for the initial rapid action of the enzyme on chromatin. Bollum[3] reports degradation of synthetic homopolymer complexes by DNase I. The intracellular functions of the enzyme are probably controlled by a DNase inhibitor[17], which according to Lazarides and Lindberg[13] is actin.

Molecular weight: 31,000.[18]

Composition: There are four deoxyribonucleases of beef pancreas: A, B, C, and D.[14, 25, 27] Five have been reported by Junowicz and Spencer.[7] They are glycoproteins differing from each other either in carbohydrate side-chain or polypeptide component.[14, 25] DNase A is the predominant form[26]; its amino acid sequence has been reported.[16]

Optimum pH: 7.8.

Extinction coefficient: $E^{1\%}_{280}$= 11.1.

Activators: DNase I is activated by bivalent metals.[8, 22, 23] Maximum activation is attained with Mg^{2+} plus Ca^{2+}. It has been indicated that a metallosubstrate, such as Mg salt of DNA might be necessary.[6, 21, 22]

Specificity: See Clark and Eichhorn[4], and Bernardi et al.[1]

Inhibitors: According to Davidson[5], citrate completely inhibits magnesium-activated but not manganese-activated enzyme. DNase I is inhibited by chelating agents such as EDTA[7,8], and sodium dodecyl sulfate.[15]

Stabilizers: The most likely proteolytic contaminant of DNase I is chymotrypsin B. Price, et. al.[24] report that DNase I can be stabilized against proteolytic digestion by 5 mM Ca^{2+}. Diisopropylfluorophosphate (DFP) may also be used to inhibit contaminating proteases.[12]

Kunitz Assay

Method: That developed by Kunitz[10,11] based upon the increased absorbance at 260 nm observed during the depolymerization of DNA by DNase. A unit causes an increase in absorbance at 260 nm of 0.001 per minute per ml when acting upon highly polymerized DNA at 25°C and pH 5.0 under the specified conditions. A standard enzyme preparation should be run in parallel with an unknown because standardization of DNA preparations and their degree of polymerization in solution is not possible.

Reagents

- 1.0 M Acetate buffer, pH 5.0
- 6.25 mM Magnesium sulfate in reagent grade water
- A standard DNase containing a defined activity of approximately 2000 DNase units.
- Highly polymerized DNA. Dissolve 10 mg DNA in 200 ml of 6.25 mM magnesium sulfate. Let stand overnight at room temperature. Add 25 ml of 1.0 M acetate buffer, pH 5.0 and dilute to a final volume of 250 ml with reagent grade water. (Substrate solution may be prepared in larger batches and stored for 2-3 weeks at 0 - 4°C.)

Enzyme

Note: Pancreatic deoxyribonuclease is unusually sensitive to physical denaturation by shaking. Mixing should be done by gentle inversion. Dissolve the standard in 1.0 ml of reagent grade water. Dilute further to a concentration of 20-60 u/ml. All dilutions are made in reagent grade water.

Sample to be assayed: Dissolve at a concentration of 1 mg/ml. Dilute further to a concentration of 20-60 u/ml immediately before the assay.

Procedure

1. Adjust spectrophotometer at 260 nm and 25°C.
2. Pipette 2.5 ml of substrate into cuvettes and incubate in spectrophotometer at 25°C for 3-4 minutes to establish blank rate if any, and to reach temperature equilibration.
3. Add 0.5 ml of diluted standard and record A260 for 8 - 10 minutes.
4. Calculate A_{260}/minute from linear portion of curve following a brief lag.

Note: The change in A_{260} for this assay is not generally linear from the initial time and is linear for only short periods. The most linear portion should be used in determining the activity. A rate of 0.008 - 0.018 α/min. is recommended.

Calculate the "factor" for the standard.

$$\text{Factor} = \frac{\text{activity of standard as stated by the vendor}}{\Delta A_{260}/\text{min} \times \text{dilution}}$$

Using the diluted sample to be tested, repeat the above procedure. Record the A_{260}/minute from the linear portion of the curve.

Calculation:

Activity is compared to that of the standard.

Units/mg = ΔA_{260} x dilution x factor

Availability:

Catalog Number	Description	Size
100574	Deoxyribonucleas I, bovine pancreas, activity ~1,000,000 Dornase units/vial	1 vial
100575	Deoxyribonuclease I, bovine pancreas, activity ~2000-2600 Kunitz units/mg dry weight. Source: Bovine Pancreas originating from New Zealand	5 mg 10 mg 20 mg 100 mg 250 mg
190062	Deoxyribonuclease I, bovine pancreas, activity ~50,000 - 150,000 Dornase units/mg solid	1 x 10^7 U 5 x 10^7 U
154022	Deoxyribonuclease I - Colloidal Gold 5 nm conjugate	0.25 ml 0.50 ml 1.0 ml
154023	Deoxyribonuclease I - Colloidal Gold 10 nm conjugate	0.25 ml 0.50 ml 1.0 ml
154024	Deoxyribonuclease I - Colloidal Gold 20 nm conjugate	0.25 ml 0.50 ml 1.0 ml

References:

1. Bernardi, A., Gaillarad, C., and Bernardi, G., "The Specificity of Five DNases as Studied by the Analysis of 5'-Terminal Doublets," *Eur. J. Biochem.*, **v. 52**, 451 (1975).
2. Billing, R., and Bonner, J., "The Structure of Chromatin as Revealed by Deoxyribonuclease Digestion Studies," *Biochim. Biophys. Acta*, **v. 281**, 453 (1972).
3. Bollum, F., "Degradation of the Homopolymer Complexes Polydeoxyadenylate-Polydeoxythymidylate, Polydeoxyinosinate-Polydeoxycytidylate and Polydeoxyguanylate-Polydeoxycytidylate by Deoxyribonuclease I," *J. Biol. Chem.*, **v. 240**, 2599 (1965).
4. Clark, P., and Eichhorn, G., "A Predictable Modification of Enzyme Specificity. Selective Alteration of DNA Bases by Metal Ions to Promote Cleavage Specificity by Deoxyribonuclease," *Biochem.*, **v. 13**, 5098 (1974).
5. Davidson, J., in *The Biochemistry of the Nucleic Acids*, **7th Ed.**, 192 (1972).
6. Douvas, A., and Price, P., "Some Effects of Calcium and Magnesium Ions on the Activity of Bovine Pancreatic Deoxyribonuclease A," *Biochim. Biophys. Acta*, **v. 395**, 201 (1975).
7. Junowicz, E., and Spencer, J., "Studies on Bovine Pancreatic Deoxyribonuclase A. I. General Properties and Activation with Different Bivalent Metals," *Biochim. Biophys. Acta*, **v. 312**, 72 (1973).
8. Junowicz, E., and Spencer, J., "Studies on Bovine Pancreatic Deoxyribonuclease A. II. The Effect of Different Bivalent Metals on the Specificity of Degradation of DNA," *Biochim. Biophys. Acta*, **v. 312**, 85 (1973).
9. Kunitz, M., "Isolation of Crystalline Desoxyribonuclease From Beef Pancreas," *Science*, **v. 108**, 19 (1948).
10. Kunitz, M., "Crystalline Desoxyribonuclease. I. Isolation and General Properties, Spectrophotometric Method for the

Measurement of Desoxyribonuclease Activity," *J. Gen. Physiol.*, **v. 33**, 349 (1950).
11. Kunitz, M., "Crystalline Desoxyribonuclease. II. Digestion of Thymus Nucleic Acid (Desoxyribonucleic Acid). The Kinetics of the Reaction," *J. Gen. Physiol.*, **v. 33**, 363 (1950).
12. Laskowski, M., Sr., "Procedures," in *Nucleic Acid Research* (Cantoni, G., and Davies, D., eds.,), 85 (1966).
13. Lazarides, E., and Lindberg, U., "Actin is the Naturally Occurring Inhibitor of Deoxyribonuclease I," *Proc. Natl. Acad. Sci. USA*, **v. 71**, 4742 (1974).
14. Liao, T., "Bovine Pancreatic Deoxyribonuclease D," *J. Biol. Chem.*, **v. 249**, 2354 (1974).
15. Liao, T., "Reversible Inactivation of Pancreatic Deoxyribonuclease A by Sodium Dodecyl Sulfate," *J. Biol. Chem.*, **v. 250**, 3831 (1975).
16. Liao, T., Salnikow, J., Moore, S., and Stein, W., "Bovine Pancreatic Deoxyribonuclease A. Complete Covalent Structure of the Polypeptide Chain," *J. Biol. Chem.*, **v. 248**, 1489 (1973).
17. Lindberg, M., and Skoog, L., "Purification from Calf Thymus of an Inhibitor of Deoxyribonuclease I," *Eur. J. Biochem.*, **v. 13**, 326 (1970).
18. Lindberg, U., "Molecular Weight and Amino Acid Composition of Deoxyribonuclease I," *Biochem.*, **v. 6**, 335 (1967).
19. Matsuda, M., and Ogoshi, H., "Specificity of DNase I. Estimation of Nucleosides Present at the 5'-Phosphate Terminus of a Limit Digest of DNA by DNase I," *J. Biochem. Japan*, **v. 59**, 230 (1966).
20. Mirsky, A., and Silverman, B., "Blocking by Histones of Accessibility to DNA in Chromatin," *Proc. Natl. Acad. Sci. USA*, **v. 69**, 2115 (1972).
21. Perlgut, L., and Hernandez, V., "Kinetic Studies on the Mechanism of Mg(II) Activation of Deoxyribonuclease I," *Biochim. Biophys. Acta*, **v. 289**, 169 (1972).
22. Poulos, T., and Price, P., "Some Effects of Calcium Ions on the Structure of Bovine Pancreatic Deoxyribonuclease A," *J. Biol. Chem.*, **v. 247**, 2900 (1972).
23. Price, P., "The Essential Role of Ca^{2+} in the Activity of Bovine Pancreatic Deoxyribonuclease," *J. Biol. Chem.*, **v. 247**, 2985 (1972).
24. Price, P., Stein, W., and Moore, S., "Effect of Divalent Cations on the Reduction and Re-formation of the Disulfide Bonds of Deoxyribonuclease," *J. Biol. Chem.*, **v. 244**, 929 (1969).
25. Salnikow, J., and Murphy, D., "Bovine Pancreatic Deoxyribonuclease A and C. A Proline for Histidine Substitution in Deoxyribonuclease C," *J. Biol. Chem.*, **v. 248**, 1499 (1973).
26. Salnikow, J., Liao, T., Moore, S., and Stein, W., "Bovine Pancreatic Deoxyribonuclease A," *J. Biol. Chem.*, **v. 248**, 1480 (1973).
27. Salnikow, J., Moore, S., and Stein, W., "Comparison of the Multiple Forms of Bovine Pancreatic Deoxyribonuclease," *J. Biol. Chem.*, **v. 245**, 5685 (1970).
28. Schmidt, G., Cashion, P., Suzuki, S., Joseph, J., Demarco, P., and Cohen, M., "The Action of Deoxyribonuclease I (Deoxyribonucleate Oligonucleotidohydrolase, EC-Number 3.1.4.5) on Calf Thymus Nucleohistone," *Arch. Biochem. Biophys.*, **v. 149**, 513 (1972).
29. Young, E., and Sinsheimer, R., "A Comparison of the Initial Actions of Spleen Deoxyribonuclease and Pancreatic Deoxyribonuclease," *J. Biol. Chem.*, **v. 240**, 1274 (1965).