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TECHNICAL INFORMATION

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 DNA²⁹, 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 Silverman²⁰, 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 Bonner² 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. Bollum³ reports degradation of synthetic homopolymer complexes by DNase I. The intracellular functions of the enzyme are probably controlled by a DNase inhibitor¹⁷, which according to Lazarides and Lindberg¹³ 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 form²⁶; 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 Eichhorn⁴, and Bernardi et al.¹

Inhibitors: According to Davidson⁵, 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

- Adjust spectrophotometer at 260 nm and 25°C.
- 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.
- Add 0.5 ml of diluted standard and record A_{260} for 8 - 10 minutes.
- Calculate A_{260}/minute from linear portion of curve following a brief lag.

Note: The change in A₂₆₀ 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 a/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 A₂₆₀/minute from the linear portion of the curve.

Calculation:

Activity is compared to that of the standard.

$$\text{Units/mg} = \text{DA}_{260} \times \text{dilution} \times \text{factor}$$

Availability:

Catalog Number	Description	Size
100574	Deoxyribonuclease 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

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