Catalog Number: 100574, 100575, 190062

Deoxyribonuclease I

CAS #: 9003-98-9

Description: Deoxyribonuclease from beef pancreas, DNase I, was first crystallized by Kunitz. 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. DNase I acts upon single chain DNA, 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, 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 suggest that DNase attacks the histone-free strand of chromatin DNA. Schmidt, et. al. indicate that hydrolysis of the histone-free region of DNA strands accounts for the initial rapid action of the enzyme on chromatin. Bollum reports degradation of synthetic homopolymer complexes by DNase I. The intracellular functions of the enzyme are probably controlled by a DNase inhibitor, which according to Lazarides and Lindberg is actin.

Molecular weight: 31,000.

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

Optimum pH: 7.8.

Extinction coefficient: E1%280 = 11.1.

Activators: DNase I is activated by bivalent metals. Maximum activation is attained with Mg2+ plus Ca2+. It has been indicated that a metallosubstrate, such as Mg salt of DNA might be necessary. It is inhibited by chelating agents such as EDTA and sodium dodecyl sulfate.

Specificity: See Clark and Eichhorn, and Bernardi et al.

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

Stabilizers: The most likely proteolytic contaminant of DNase I is chymotrypsin. Price, et. al. report that DNase I can be stabilized against proteolytic digestion by 5 mM CaCl2. Disopropylfluorophosphate (DFP) may also be used to inhibit contaminating proteases.

Kunitz Assay

Method: That developed by Kunitz 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 A260 for 8 - 10 minutes.
– Calculate A260/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 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 $A_{260}$/minute from the linear portion of the curve.

**Calculation:**
Activity is compared to that of the standard.

Units/mg = DA$_{260}$ x dilution x factor

**Availability:**

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

**References:**

– Matsuda, M., and Ogoshi, H., "Specificity of DNase I. Estimation of Nucleosides Present at the 5'-Phosphate Terminus of a