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Deoxyribonuclease II Assay

Method

That developed by Kunitz (1950) based upon the increase in absorbance at 260 nm during the hydrolysis of DNA. The method has been adapted from that used for DNase I by reducing the pH to 4.6 and lowering the magnesium concentration to 0.001 M. One unit causes an increase in absorbance at 260 nm of 0.001 per minute per ml at 25°C and pH 4.6 under specified conditions. Bernardi (1971) discusses the relationship of absorbance units so defined to terminal phosphate formation, i.e., substrate bonds hydrolyzed.

Reagents

- 1.0 M Acetic acid, pH 4.6
- 0.02 M Magnesium sulfate

Substrate: Dilute 12.5 ml of 0.02 M magnesium sulfate to a volume of 200 ml with reagent grade water. Add 10 mg calf thymus DNA. Let stand overnight at room temperature. Add 25 ml of 1.0 M acetic acid, pH 4.6 and adjust the pH to 4.6. Dilute to a final volume of 250 ml with reagent grade water. Store at 2 - 8°C.

Enzyme

Dissolve enzyme at 1 mg/ml in reagent grade water. Dilute further in water immediately prior to use to a concentration of 50 - 150 units/ml.

Procedure

Adjust the spectrophotometer to 260 nm and 25°C.

Pipette 2.5 ml of substrate into cuvette and incubate in the spectrophotometer for 3 - 4 minutes to establish blank rate, if any, and to reach temperature equilibrium. Add 0.5 ml diluted enzyme. Record increase in A_{260} from linear portion of curve. 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 change in A_{260} should be determined from the portion which is most linear.