Deoxyribonuclease, Enzyme Activity

Catalog	Unit
TBP0066-1G	1 g
TBP0066-5G	5 g

Product Details

Form: Freeze-dried powder

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 200 U/mg protein

Protein: 90%

Unit Definition

The amount of enzyme which results in an increase in absorbance at 260 nm and 0.001 per minute per ml at 25°C at pH 5.00.

Assay Method

The assay method is a modification of the method of Kunitz. An increase in absorbance at 260 nm, caused by the depolymerization of DNA by the enzyme, is a measure of enzyme activity.

Applications

Deoxyribonuclease (DNase I) (EC 3.1.4.5), from bovine pancreas, is an endonuclease catalyzing the hydrolysis of phosphodiester linkages, adjacent to a pyrimidine nucleotide yielding 5'phosphate terminated polynucleotides. The enzyme is able to act upon single as well as double-stranded DNA and on chromatin.

The DNase from bovine pancreas exists in multiple forms with DNase A being the predominant form. In addition to bovine pancreas, DNase activity has also been demonstrated in other mammalian tissues.

Reagents

1. 1.00 M Acetate Buffer, pH 5.00.
2. 0.05 M Magnesium sulfate.
3. Substrate: 4 mg DNA/100 ml solution. (Dissolve 4 mg of highly polymerized DNA in 50 ml of distilled water, add 10 ml acetate buffer and 10 ml magnesium sulfate. Dilute to 100 ml with distilled water.) The substrate will be stable for 2 weeks if stored at 4°C.
4. Enzyme - dissolved in acetate buffer to yield a final concentration of 40-60 U/ml.

Procedure

1. Set spectrophotometer (equipped with a strip chart recorder and temperature control) at 260 nm and 25°C.
2. In quartz cuvettes pipette the following at 25°C:

	TEST	CONTROL
Substrate	3.0 ml	3.0 ml
Distilled water		1.0 ml
Enzyme @ zero time	1.0 ml	

Record the rate of increase in absorbance at 260 nm for approximately 8 min. after the initial lag time of approximately 15 min.

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{260nm/min})(1000)}{(\text{mg Enz./ml})}$$

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Tribioscience, Inc.; 365 San Aleso Ave, Sunnyvale, CA 94085 Phone: 408-498-0197
info@tribioscience.com; www.tribiosciences.com