FT-24354

DNase I

Description

Catalog #: Partially purified. A lyophilized powder.

243545	25 mg
243546	100 mg

Chromatographically purified. A lyophilized powder with glycine as a stabilizer.

243540	20 mg
243541	100 mg

Deoxyribonuclease I, Ribonuclease & Protease Free
Molecular Biology Grade. Chromatographically purified to remove RNase and protease. Lyophilized in vials. Each 10,000 unit vial contains 2 mg glycine, 2µmoles calcium, and ≥10,000 units of DNase I. Each 2,500 unit vial contains 0.5mg glycine, 0.5µmoles calcium, and ≥2,500 units of DNase I. Dissolving the entire 10,000 unit vial in 5 ml, or the entire 2,500 unit vial in 1.25 ml, provides the equivalent of a 1 mg/ml solution. (ku = 1000un)

T06510	2500 units
T06511	10000 units

Deoxyribonuclease I, Ribonuclease & Protease Free, Solution
Molecular Biology Grade. Chromatographically purified to remove RNase and protease. Supplied as a solution at approximately 2 Kunits /ml (approximately 1 mg/ml) containing 50% glycerol and 1mM calcium chloride.

18405D	100 units
18405F	500 units

Name: **Deoxyribonuclease I, Bovine Pancreas**
2,000 Units/mg

Unit Definition 1 unit causes an increase in absorbance at 260nm of 0.001 per minute per ml at 25°C when acting upon highly polymerized DNA at pH 5.0. **Note**: Kunitz units as reported by other suppliers can be 2 to 4 times higher than Kunitz units as measured at InterBiotech. As measured at InterBiotech, 0.005 Kunitz unit digests 1µg of lambda DNA in 10 minutes at 37°C in 50mM Tris, 1mM Mg^{2+}, pH 7.8 in a 50µl reaction. Correlation of digestion units with Kunitz units is different for other DNA and buffer systems.

Storage: +4°C or –20°C for long term storage and solution

For Research Use Only

Introduction

Bovine pancreatic deoxyribonuclease is an endonuclease which splits phosphodiester linkages, preferentially adjacent to a pyrimidine nucleotide yielding polynucleotides with free hydroxyl group at the 3' position and phosphate group at the 5' position. The average chain length of a limit digest is a tetranucleotide.

Molecular Biology Grade means that the Dnase I are suitable for use in techniques requiring digestion of DNA in the recovery of intact RNA or where the integrity of structural proteins or enzymes must be maintained. Applications have included nick translation, DNA mapping, isolation of nuclear RNA and protein, plasmid construction, and RNA polymerase synthesis of RNA probes and RT-PCR.

Technical and Scientific Information

Deoxyribonuclease from beef pancreas, DNase I is an endonuclease, splitting 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. 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. DNase attacks the histone-free strand of chromatin DNA. The hydrolysis of the histone-free region of DNA strands accounts for the initial rapid action of the enzyme on chromatin. The intracellular functions of the enzyme are probably controlled by actin , a DNase inhibitor.

Characteristics of the Enzyme from Bovine Pancreas:

Stability/Storage: When properly stored, all grades of deoxyribonuclease are stable for 2-3 years. Recommended storage temperature for all grades of DNases is 2-8°C. Product code 18405D/F may be stored at -20°C. For long term storage in solution, Product code 243540/1 may be dissolved in 5mM acetate, 1mM calcium, pH 4.5 and stored in single use aliquots at -20°C or -70°C for up to one year. Only freeze and thaw once; thawed aliquots are stable refrigerated at least several weeks. Addition of 50% glycerol will maintain a liquid state at -20°C without affecting stability and material in 50% glycerol can be removed and returned to -20°C repeatedly. Product code T06510/1 is unusually stable due to the absence of protease. For long term storage of product code T06510/1 after reconstitution, use water or any buffer pH 4.0 to 9.0 except phosphate; avoid calcium chelators; add 50% glycerol for storage as liquid at -20°C; aliquot in single use containers; only freeze and thaw once; thawed aliquots are stable refrigerated at least several weeks.

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 (1973a). They are glycoproteins differing from each other either in carbohydrate side-chain or polypeptide component. DNase A is the predominant form.

Optimum pH: 7.8.

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

Activators: DNase I is activated by bivalent metals. 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.

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

Stabilizers: The most likely proteolytic contaminant of DNase I is chymotrypsin B. DNase I can be stabilized against proteolytic digestion by 5 mM Ca^{2+}. Diisopropylfluorophosphate (DFP) may also be used to inhibit contaminating proteases.

Stability: The lyophilized enzyme is stable for 2-5 years when stored at 5°C.

Assay

Method: That developed by Kunitz (1950) 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
- Standard DNase Vial (Code:18405G) containing a defined activity of approximately 2000 DNase units per vial.
- Highly Polymerized DNA (Code: 43353A). 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 vial in 1.0 ml of reagent grade water. Care must be taken when opening the vial that no lyophilized material is lost. This solution will contain the number of units/ml as stated on the label. 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 25deg.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 $\geq A_{260}$/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 ≥[[Alpha]]/min. is recommended.

Calculate the "factor" for the standard vial.

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

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

Calculation:

Activity is compared to that of the standard vial.

$$\text{Units/mg} = \Delta A_{260}/\text{minute} \times \text{dilution} \times \text{factor}$$

Deoxyribonuclease activity can also be conveniently measured in a radial diffusion assay system.

Related / associated products

- Standard DNase Vial 18405G
- Highly Polymerized DNA 43353A

For in vitro R&D use only

Please contact InterBioTech – Interchim for any other information