FT-35601A

Nuclease S1

Product Description

Name: **Nuclease S1**

Reference: 35601A, 10000 Units 35601B, 50000 Units

Source: *Aspergillus oryzae*
Chromatographically purified. Specific for single-stranded DNA (ssDNA) degradation. Activity on native (ds) DNA undetectable under the assay conditions. A frozen solution in 30mM sodium acetate, pH 4.6, 50mM NaCl, 1mM $ZnCl_2$, and 50% glycerol.

Unit Definition: One Unit hydrolyzes one microgram of denatured calf thymus DNA per minute at 37°C, pH 4.6.

Molecular Weight: Approximately 32,000 - 36,000 daltons, exists as a monomer

Optimum pH : 4.0 - 4.6

Activators: Zn^{2+} and/or Ca^{2+}

Inhibitors: EDTA, citrate and a high concentration of SDS

Storage: –20°C

Introduction

Nuclease S1, isolated from certain *Neurospora* and *Aspergillus* species, specifically hydrolyzes both terminal and internal phosphodiester bonds of single-stranded DNA and RNA. It is used to eliminate non-annealed polynucleotide tails and hair-pin loops in DNA-RNA or DNA-DNA duplexes in hybridization studies and in genetic recombination experiments.

Directions for use

Handling and Storage

For long term storage in solution, for up to six months, dilute NUCSI to ≥6000 u/ml in water and freeze in aliquots. Dilute solutions can be stabilized by adding 0.1% albumin (Worthington Code: BSANF) and 10% glycerol.

Protocol

One unit is the amount of enzyme liberating 1µg (0.033 A260) of acid-soluble nucleotides from heat-denatured DNA per minute at 37ÁC and at pH 4.6

Reagents

Buffer: 0.2M NaCl, 0.002M $ZnCl_2$, 0.06M CH_3COONa, pH 4.6: dissolve 5.844 gms NaCl (MW 58.44), 136 mg $ZnCl_2$ (MW 136.29) and 1.85 ml concentrated glacial acetic acid in 450 ml reagent grade water. Adjust pH to 4.6 with 10M NaOH. Bring to a final volume of 500 ml with reagent grade water.

Enzyme Diluent: Dissolve 40 mg BSA in 200 ml Buffer

Substrate: Shred into small fibers, 60 mg calf thymus DNA and dissolve in 50ml reagent grade water by standing at room temperature for at least 18 hours. Additional stirring may be necessary to effect soluiton. Remove 10ml DNA solution to 10 ml of buffer. This is native calf thymus DNA solution (Substrate B)

Heat the remaining DNA solution in a large Pyrex test tube with a stir bar in boiling water on a heater/stirrer while stirring for 20 minutes. Immediately pour into **PRE-FROZEN** 1 liter beaker on ice. Mix equal volumes of the DNA

solution and ***cold*** buffer. This is heat-denatured calf thymus DNA solution (Substrate A). Use as soon as possible to prevent the blank from elevating.

15% Perchloric Acid: Add 21.5 ml concentrated perchloric acid (70%) to 78.5 ml deionized water.

Procedure

1. To clean glass tubes (two for each point) add 2ml Substrate B for tests. Include 2 tubes with 2ml substrate B for blanks (no enzyme added) and 2 tubes with 2ml Substrate A (native DNA test).
2. Incubate for 5 minutes before adding the enzyme.
3. Add 0.1ml enzyme dilution
4. Incubate at 37°C for 10 minutes.
5. Stop reaction by adding 2ml 15% perchloric acid.
6. Leave on ice for 10 minutes.
7. Centrifuge on a bench-top centrifuge for 15 minutes at 2000 rpm.
8. Withdraw 3ml supernatant and read A260.

Calculation

$$\text{units / ml} = \frac{\text{A260 of sample} - \text{A260 of blank} \times \text{dilution} \times 1242}{10}$$

Where 1242 is a factor derived by dividing the reaction volume, 4.1ml, by the A260 of 1µg, which is 0.033, and dividing by the enzyme sample volume used, which is 0.1ml.

References

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