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## **Certificate of Analysis**

Product Name: DNase I, Pharmaceutical Grade

Cat No : DN102 Lot No : 10062312

TEST	Result
Product Type	Native Enzymes
Source	Bovine Pancreas
CAS No	9003-98-9
Form	Lyophilized Powder
Activity	9,580 Kunitz units per mg dry weight

**Description**: Deoxyribonuclease I (DNase I) is an endonuclease that hydrolyzes double-stranded or single-stranded DNA preferentially at sites adjacent to pyrimidine nucleotides. The product of hydrolysis is a complex mixture of 5'-phosphate mononucleotides and oligonucleotides. In the presence of magnesium ion, DNase I attacks each strand of DNA independently and the cleavage sites are random. In the presence of manganese(II), DNase I cleaves both strands of DNA at approximately the same site. Most protocols use magnesium ion with DNase but for specific purposes, manganese is cited. Supplied as a lyophilized powder. Purified precristalline DNase. All products from animal sources are produced from starting material of North American origin, collected in United States Department of Agriculture (USDA) approved facilities and inspected to be free of disease.

Unit Definition: One unit causes an increase in Absorbance at 260nm of 0.001 per minute per ml.

At 25°C, pH5.0 when acting on highly polymerized DNA in the presence of ionized magnesium and calcium.

**Note**: Kunitz units as reported by other suppliers can be 2 to 4 times higher than Kunitz units as measured at Worthington. As measured at Worthington, one Kunitz unit digests  $1\mu g$  of lambda DNA in 10 minutes at  $37^{\circ}C$  in 50mM Tris, 1mM  $Mg^{2+}$ , 1mM  $Ca^{2+}$ , pH 7.8 in a  $50\mu l$  reaction. Correlation of digestion units with Kunitz units is different for other DNA and buffer systems.

Optimum pH for activity: 7-8

**Thermal stability:** A protease-free DNase is stable at pH 5-7 up to 60 °C for at least five hours; at 62 °C, a 1 mg/mL solution lost activity at 6% per hour in either acetate buffer (pH 5) or Tris buffer (pH 7.2). Activity was destroyed at 68 °C. (The same research indicated that a solution at 0.1 mg/mL showed no activity loss after five hours at 62 °C.)

**Activators**: DNase I has an absolute requirement for divalent metal cations. The most commonly used is magnesium(II); however, Mn(II), calcium, cobalt and zinc also activate DNase I. [Ca2+] at 5 mM will stabilize DNase I against proteolytic digestion; 0.1 mM is needed to reduce the rate of inactivation by one-half.

**Inhibitors**: β-mercaptoethanol (the reduced enzyme is inactive, but can be reactivated in presence of Ca or Mg); chelators; sodium dodecyl sulfate (SDS); actin. There is no general inhibitor specific for DNase I.

Storage/Stability: Under -20°C. Keep dry. Warm to room temperature before opening.

DNase I is properly lyophilized formulation maintaining adequate physical and chemical stability of the protein during shipping and long-term storage, even at ambient temperatures.

When properly stored, it is stable for 2-3 years, recommended storage temperature for DNases I is -20°C. For long term storage in solution, 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 -20°C without affecting stability and material in 50% glycerol can be removed and returned to -20°C repeatedly. For long term storage of DPRF 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 -20°C; aliquot in single use containers; only freeze and thaw once; thawed aliquots are stable refrigerated at least several weeks.

Caution: Avoid contact with eyes, skin and clothing.

Wash all areas of contact with copious amounts of water. See Material Safety Data Sheet for additional information. For laboratory or further manufacturing use only.

Not intended for household use.

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