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ProductInformation

DEOXYRIBONUCLEASE I
from Bovine Pancreas

Product Number **D4527, D4263, D4513, D5025, DN-25, AND DN-EP**
Storage Temperature –20 °C

CAS #: 9003-98-9
EC #: 3.1.21.1
Synonyms: DNase I; Deoxyribonucleate 5'-oligonucleotidohydrolase; Deoxyribonuclease A

Sigma offers DNase I in several purities and in special packaging for convenience. General physical properties are listed in the catalog. Please also see the Molecular Biology special reagents for a DNase I that is warranted as RNase-free.

Product Description
Appearance: white powder, occasionally off-white or with light yellow cast. Only DN-25 is sometimes a light tan powder.[1]
Molecular weight: 30,072 calculated from sequence[2]
Structure: a single polypeptide containing two disulfide bridges and a single carbohydrate moiety of 7 monosaccharides.[2]

Bovine pancreatic DNase exists as four isozymes, having isoelectric points for A, B, C and D: 5.22, 4.96, 5.06 and 4.78.[3] The predominant form is A, with smaller amounts of B and C, and only minor amount of D. Details of structural differences have been reported.[4] Sigma's method of isolation does not select for any isozyme.[5]

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.[11] Most protocols use magnesium ion with DNase I[6] but for specific purposes, manganese is cited.[12]

DNase has been used to introduce random nicks into double-stranded DNAs in preparation for radiolabeling by nick translation or to introduce a single nick into circular DNAs in preparation for resection. Protocols for digestion of DNA using DNase and for removal of RNase from DNase preparations are described.[6,11]

Optimum pH for activity: 7-8[6]
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.)[8]
Activators: DNase I has an absolute requirement for divalent metal cations. The most commonly used is magnesium(II)[1,6]; however, Mn(II), calcium, cobalt and zinc also activate DNase I.[6,7] [Ca^{2+}] at 5 mM will stabilize DNase I against proteolytic digestion; 0.1 mM is needed to reduce the rate of inactivation by one-half.[8]
Inhibitors: β-mercaptoethanol (the reduced enzyme is inactive, but can be reactivated in presence of Ca or Mg[7]); chelators; sodium dodecyl sulfate (SDS)[9]; actin.[10] There is no general inhibitor specific for DNase I.[6]

Preparation Instructions
Not all DNase I products are tested for solution appearance, but those which have specific assays are tested in 0.15 M NaCl at 5 mg/mL; clear solutions are seen for all but DN-25, which may give a hazy solution. Solution stability depends on several factors: stabilizing calcium ion, concentration, temperature and protease contamination. Most of Sigma DNases already contain calcium chloride to stabilize them to protease digestion.[8] When DNase I was dissolved in 0.15 M NaCl at 10 mg/mL, stored for a week, a frozen aliquot (-20 °C) lost <10% activity, whereas a refrigerated aliquot (2-8 °C) lost about 20% activity.[5] Magnesium or other divalent cation is needed for activation.

Solutions of DNase I are stable for several hours at 60 °C, but rapidly denature at 68 °C (as previously noted). A working solution of Dnase I standard (Product No. D 4263) should kept on wet ice and only warmed when it is to be used. The standard solution should be discarded after eight hours. Solutions containing at least 1 mg protein/mL with 5 mM calcium chloride and stored at -20 °C and should retain >90% activity for at least a year. Solutions containing <0.1 mg protein/mL are considerably less stable; they may require gelatin as stabilizer.[13]

For applications unaffected by glycerol, two storage buffers have been recommended. These solutions do not freeze at –20 °C and are used for Sigma's molecular biology-tested DNase I products.

a. 20 mM sodium acetate pH 6.5, containing 5 mM $CaCl_2$ and 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 50% (v/v) glycerol; protein at least at 5 mg/mL.[5]
b. 10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 50% (v/v) glycerol; protein at least 2 mg/mL.[6]

Removal of DNA from preparations can be accomplished by incubation with 20-50 µg/ml DNase I (no specific activity given) in the presence of 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$ at 37 °C for 60 minutes.[6]

Unit Definition: One Kunitz unit will produce a ΔA_{260} of 0.001 per minute per mL at pH 5.0 at 25 °C, using DNA (D 1501 or D 1626) as substrate, with $[Mg^{2+}]$ = 4.2 mM.

Storage/Stability
When stored dry and frozen D 4513, D 4527, D 4263, and DN-EP have a shelf-life of three years. D 5025 has a shelf-life of two years and DN-25 has a shelf-life of four years.

References

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