DNase I lyophilized powde10 mg

For research use only

Cat No: YT9058
Size: 10 mg
Store at –20°C

Product Description

Deoxyribonuclease I (DNase I) is an endonuclease that cleaves single and double-stranded DNA by preferentially acting on phosphodiester bonds adjacent to pyrimidines, to produce polynucleotides with terminal 5`-phosphates. In the presence of Mg2+ ions, DNase I attacks each strand of DNA independently and the cleaves randomly. If Mn2+ ions are present, both DNA strands are cleaved at approximately the same site. DNase I is used to remove DNA from protein and nucleic acid samples, and to nick DNA as a first step to incorporate labeled bases into DNA.
The vial of DNase may appear to be empty. This is due to lyophilized enzyme sticking to the septum. To avoid loss of DNase befor opening the vial,spin it.

Unit Definition: One Kunitz unit will produce a change in A260 of 0.001 per minute per mL at pH 5.0 at 25 °C, using DNA, Type I or III, as substrate with [Mg2+] = 4.2 mM.
This enzyme assay reaction is performed in 95 mM acetate buffer, pH 5.0, at 25 °C, containing 4.75 mM Mg2+ and 1.9 mM Ca2+, in a 3 ml reaction.

Preparation Instructions:

For preparation of DNase I, add 2ml 0f 0.15M NaCl in 10mg/ml (6000u/ml) DNase I stock, the final concentration will be 5mg/ml (3000u/ml).
For applications unaffected by glycerol, two other storage buffers are options, as these formulations do not freeze at ‑20 °C.
1. 20 mM sodium acetate (pH 6.5), containing 5 mM CaCl2 and 0.1 mM phenyl methyl sulfonyl fluoride (PMSF), 50% (v/v) glycerol, with DNase I at ≤5 mg/mL.
2. 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM MgCl2, 1 mM DTT, 50% (v/v) glycerol, with DNase I at ≤2 mg/mL.

Removal of genomic DNA from RNA preparations

1. use one unit of DNase I per 1 to 5 μg of total RNA in a 50 μl total volume
2. Incubate 20 minutes at +25 to +37°C
3. inactive DNase I

Inactivation DNaseI:

1-Heat inactivation: Probably the most common method of DNase inactivation is heat treatment, typically for 5 minutes at 75°C. Although this method appears straightforward, the divalent cations in the DNase digestion buffer can cause (chemically-induced) strand scission of RNA when heated.

2-Proteinase K treatment and organic extraction: Proteinase K treatment followed by phenol:chloroform extraction is probably the most rigorous method for DNase inactivation and removal, but it is time-consuming, and organic extractions often cause some sample loss. Sample loss can be minimized by back extraction of the phenol:chloroform phase, but this adds another step to an already time-consuming procedure. Additionally, many people prefer to avoid working with hazardous phenol.

3-EDTA chelation of cations: The addition of EDTA to DNase digestion reactions chelates ions in the digestion buffer, that are required for DNase I activity. The DNase I can then be safely heat inactivated without loss of RNA. Add of 1 µL of 25 mM EDTA solution to the reaction mixture in 10 µL reaction with 1 unit DNase I, Amplification Grade (or 1:1 molar ratio of Mg++ ions:EDTA) to chelate the Mg++ ions in the DNase I buffer. Heat for 10 min at 65 °C.

Additional information:

Activators:

DNase I has an absolute requirement for divalent metal cations. The most commonly used Mg2+. However, Mn2+, Ca2+, Co2+, and Zn2+ will activate DNase I. A concentration of 5 mM Ca+2 will stabilize DNase I against proteolytic digestion; 0.1 mM is needed to reduce the rate of inactivation by one-half.

Inhibitors:

2-Mercaptoethanol (the reduced enzyme is inactive, but can be reactivated in the presence of Ca2+ or Mg2+ ions); chelators; sodium dodecyl sulfate (SDS); and actin. There is no general inhibitor specific for DNase I. Citrate inhibits Mg2+-activated DNase I, but not Mn2+-activated DNase I.