

DNase I

Deoxyribonuclease I [E.C. 3.1.21.1]

Product No. A3778

Description

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| Source: | bovine pancreas |
| Molecular weight: | approx. 31000 g/mol |
| CAS-No.: | [9003-98-9] |
| HS-No.: | 35079090 |
| Activity: | min. 3000 U/mg (Kunitz) |
| supplied as: | salt-free, freeze-dried powder |
| Solubility: | Dissolves readily at 5 mg/ml in 150 mM NaCl to give a clear, colorless solution |
| Storage: | -20°C |
| Unit definition (Kunitz): | One unit is defined as the amount of enzyme which causes an increase of absorbance at 260 nm of 0.001 per minute at 25°C and pH 5.0 based on the method of Kunitz. |

Comment

Deoxyribonuclease I (DNase I) from beef pancreas is an endonuclease (glycoprotein), which preferentially cleaves the phosphodiester bond in the DNA behind pyrimidine nucleotides. This results in a polynucleotide with a 5'-phosphate and a free OH-group in position 3'. DNase I cleaves single-stranded and double-stranded DNA as well as chromatin. The specificity of the enzyme reaction (single-strand-'Nicks' versus double-strand breakes) is determined by the ions available. In the presence of Mg²⁺ single-strand nicks are generated and in the presence of Mn²⁺ double-strand breakes. The pH-optimum of DNase I is 7.8 and it is activated by divalent cations. Maximum activation requires the presence of Mg²⁺ and additional Ca²⁺. Calcium ions (5 mM) protect DNase I from proteolytic digest. Inhibition is achieved by citrate, if activation is done by magnesium, but not if manganese has been the activator. Besides, it is inhibited by chelators such as EDTA and SDS or β -mercaptoethanol.

The enzyme is used in molecular biology techniques like digestion of DNA, in the RNA purification (ref. 2 Suppl. 1 pp. 4.1.4) or generating "random nicks" for "nick translation" (ref. 2 Suppl. 9 pp. 3.5.4-6) or 'footprint'-assays (ref. 2 Suppl. 7 chapter 12.4) or investigations on chromatin (ref. 2 Suppl. 48 chapter 21.4.1).

DNase I is readily soluble in e. g. 0.15 M sodium chloride or in reaction buffer (e. g. 50 mM Tris · Cl, pH 7.5; 10 mM MgCl₂ (single-strand 'nicks') and 10 mM MnCl₂ (double-strand breakes), respectively; 50 μ g/ml BSA; ref. 2 Suppl. 8 page 3.12.5). For storage dissolve DNase I in 50 % glycerol (w/v); 20 mM Tris · Cl, pH 7.5; 1 mM MgCl₂. For stability reasons the concentrations should be at least 1 mg/ml. Do not vortex. This solution is stable for more than one year (ref. 2 Suppl. 8 page 3.12.5). The lyophilized form is stable for 2 - 5 years if kept at +4°C. If a solution is protease-free, DNase I will not loose significant activity at pH 5 - 7 and 62°C for 5 hours. The enzyme may be heat-inactivated (10 minutes at 99°C).

RNase-free DNase I: Dissolve DNase I at 1 mg/ml in 0.1 M iodoacetic acid plus 0.15 M sodium acetate at a final pH of 5.3. The solution is then heated 40 minutes at 55°C and cooled. Finally, 1 M CaCl₂ is added to the solution to 5 mM. Store frozen in small aliquots (according to ref. 2 page 3.12.6 Supplement 8).

Literature

- (1) Sambrook, J. & Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd Edition. pp. A4.40-42. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- (2) Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl, K. (eds.) 2001. *Current Protocols in Molecular Biology*. John Wiley & Sons, N.Y.
- (3) McDonald, M.R. (1955) *Methods Enzymol.* **2**, 437-447 Deoxyribonucleases
- (4) Campbell, V.W. & Jackson, D.A. (1980) *J. Biol. Chem.* **255**, 3726-3735 The effect of bivalent cations on the mechanism of DNase I.
- (5) Meinkoth, J. & Wahl, G.M. (1987) *Methods Enzymol.* **152**, 91-94 Nick-Translation.
- (6) Cobianchi, F. & Wilson, S.H. (1987) *Methods Enzymol.* **152**, 94-110 Enzymes for modifying and labeling of DNA and RNA.

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