Specification

DNase I A3778

Physical Description:	Solid
Product Code:	A3778
Product Name:	DNase I
Short Description:	delivery form: salt-free, freeze-dried powder, chromatographically prepared
Specifications:	Activity (Kunitz): min. 3000 U/mg
WGK:	1
Storage:	-20°C
Origin:	from bovine pancreas
M:	~31000 g/mol
CAS:	9003-98-9
EINECS:	232-667-0
CS:	35079090

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Specification

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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 Mg^{2+} single-strand nicks are generated and in the presence of Mn^{2+} 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 Mg^{2+} and additional Ca^{2+}. 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).**Unit definition**\: One unit is defined as that amount of enzyme which causes an increase of absorbance at 260 nm of 0.001 per minute at 25°C based on the method of Kunitz. 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 $MgCl_2$ (single-strand 'nicks') and 10 mM $MnCl_2$ (double-strand breakes), respecitvely; 50 µg/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 $MgCl_2$. For stability reasons the concentrations should be at least 1 mg/ml (The maximum solubility is 10 %). 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 $CaCl_2$ is added to the solution to 5 mM. Store frozen in small aliquots (according to ref. 2 page 3.12.6 Supplement 8).

Bibliography

(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. *Currrent Protocols in Molecular Biology*. John Wiley & Sons, N.Y. (3)McDonald, M.R. (1955) *Methods Enzymol*. **2**, 437-447Deoxyribonucleases (4)Campbell, V.W. & Jackson, D.A. (1980) *J. Biol. Chem.* **255**, 3726-3735The effect of bivalent cations on the mechanism of DNase I. (5)Meinkoth, J. & Wahl, G.M. (1987) *Methods Enzymol*. **152**, 91-94Nick-Translation. (6)Cobianchi, F. & Wilson, S.H. (1987) *Methods Enzymol*. **152**, 94-110Enzymes for modifying and labeling of DNA and RNA.

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