RNase A (DNase-free)

Ribonuclease A from bovine pancreas; salt-free, freeze-dried
Product No. A3832

Description

Molecular weight:	~13700 g/mol
CAS-No.:	[9001-99-4]
Activity:	90 U/mg (Kunitz)
DNases / Proteases:	not detectable
Storage:	-20°C
recommended stock solution:	1 - 10 mg/ml in Tris buffer
recommended working solution:	0.1 - 10 µg/ml

Ribonuclease A (RNase A) is an endoribonuclease, that specifically cleaves single-stranded RNA 3' to pyrimidine residues (cytosine, uracil). Thereby, it generates pyrimidine-3'-phosphate or oligonucleotides with terminal pyrimidine-3'-phosphates. The pH-optimum is in the range of 7.0 - 7.5. RNase A is used for the purification of RNA-free DNA, for the removal of non-hybridized regions of RNA : DNA-hybrides or as a molecular weight marker. The enzyme is inhibited by diethyl pyrocarbonate (DEPC), guanidinium salts (4 M GuaSCN), β-mercaptoethanol, heavy metals, vanadyl-ribonucleoside-complexes, RNase-inhibitor from human placenta and competitively by DNA, respectively. Regarding the latter, the effect of denatured DNA is higher than by native nucleic acids. Nevertheless, RNase A is very active under very different conditions and difficult to inactivate. At low salt-concentrations (up to 100 mM NaCl), RNase A cleaves single- and double-stranded RNA and RNA in RNA : DNA- hybrides. Under high salt concentrations (>300 mM NaCl) single-stranded RNA is cleaved only. To remove the enzyme from samples, it has to be digested by proteinase K (frequently, SDS at a final concentration of 0.6 % is added) and several phenol extractions are required. (Applications: Enzymatic manipulation of DNA and RNA: ref. 1 Suppl. 8 p. 3.13.1; minipreps of plasmid-DNA: ref. 1 Suppl. 24 p. 1.6.6; *in situ*-hybridisation of cellular RNA: ref. 1 Suppl. 7 p. 14.3.8; removal of RNA from plasmid preparations: ref. 2 p. 1.51)

Stock solutions are prepared at concentrations from 1 - 10 mg/ml in 10 mM Tris · HCl, pH 7.5; 15 mM NaCl or in 10 mM Tris · HCl, pH 7.5; 1 mM EDTA, pH 8.0 (TE buffer). The recommended working concentration is 10 µg/ml (removal of RNA from plasmid preparations; 1 hr, RT) or 100 ng/ml (preparation of 'blunt ends' of double-stranded cDNA).

Unit-definition: One unit of activity is defined as that amount of enzyme which causes the hydrolysis of RNA to yield a velocity constant, k = 1, at 25°C and pH 5.0 (Kunitz-Unit).

Stability: RNase A aggregates during lyophilizing and storage. It has a high affinity to glas surfaces, which has to be taken into consideration. At neutral pH (e. g. in PBS pH 7.4) and high concentrations (> 10 mg/ml) the enzyme will precipitate. At +4°C (lyophilized) it is stable for several years (dry storage), in solution (-20°C) several years or (+4°C) several weeks.

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