Specification

RNase A A2760

Synonym	Ribonuclease A
delivery form	salt-free, freeze dried
origin	from bovine pancreas
M	~13700 g/mol
CAS-No.:	9001-99-4
HS-No.:	35079090
EC-No.:	232-646-6
Storage:	-20°C
LGK:	10 - 13
WGK:	nwg
Specification	
Assay	approx. 70 %
Activity	min. 70 U/mg (Kunitz)

Literature

(1) Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl, K. (eds.) (1995) *Current Protocols in Molecular Biology. Page 3.13.1 Suppl. 8;* Greene Publishing & Wiley-Interscience, New York

(2) Sambrook, J. & Russel, D.W. (2001) *Molecular Cloning:* A Laboratory Manual, 3rd Edition Page A4.39. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

(3) Melton, D.A. *et al.* (1984) *Nucleic Acids Res.* **12**, 7035-7056 Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing the SP6 promoter.

(4) Winter, E. *et al.* (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7575-7579 A method to detect and characterize point mutations in transcribed genes.

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Comment

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; *inSitu*-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).
Inactivation of DNase activity: A protocol (ref. 2) suggests to dissolve 10 mg/ml RNase A in 0,01 M Sodium acetate (pH 5,2), to heat to 100°C for 15 minutes in a water bath and to cool down to room temperature very slowly. The pH value is equilibrated by adding 0.1-fold the volume of 1 M Tris-Cl (pH 7,4). **Caution:** Heating solutions of RNase A to inactivate DNase may not be satisfactory since RNase activity may be lost if precipitate formation occurs. For applications that require DNase-free RNase A we recommend our product A3832, RNase A (DNase-free).
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.