

Description:

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.

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 μ g/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.

Quality: for molecular biology

Molecular weight: ~13700 g/mol

Origin From: bovine pancreas

Delivery form: salt-free, freeze dried

Storage: -20°C

Product	Rnase A (Dnase-free)
Quality	For molecular biology
Synonym	Ribonuclease
Molecular weight	~13700 g/mol
CAS number	9001-.99-4
HS number	35079090
Origin	Form bovine pancreas
Delivery form	Salt free– freeze dried
Storage	-20°C

Parameter	Specification
DNases	Not detectable
Proteases	Not detectable
Activity	Min. 80 U/mg

Order Information

Prod. No.	Description	Quantity
4909-050	RNase A	50 mg
4909-250	RNase A	250 mg
4909-1000	RNase A	1000 mg