

Ribonuclease A

RNase A (E.C. 3.1.27.5)

Ribonuclease A (E.C. 3.1.27.5) from bovine pancreas (salt free, freeze dried)

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Product	Cat#	Package size
RNase A, min. 70 U/mg (Kunitz)	S5231.0100	100mg
RNase A, min. 70 U/mg (Kunitz)	S5231.0500	500mg
RNase A, min. 70 U/mg (Kunitz)	S5231.1000	1g

Product 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-optimium is in the range of 7.0 to 7.5. RNase is used for the purification of RNA-free DNA, for the removal of non-hybridised regions of RNA : DNA hybrides or as a molecular weight marker. The enzyme is inhibited by diethyl pyrocarbonate (DEPC), guanidinium salts (4M 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 100mM 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 (M3036, M3037) (frequently, SDS at a final concentration of 0.6% is added) and several phenol extractions are required.

Applications

Enzymatic manipulation of DNA and RNA - Minipreps of plasmid DNA - In-situ hybridisation of cellular RNA - Removal of RNA from plasmid preparations

Stock solutions

Recommended stock solutions are: from 1 - 10mg/mL in 10mM Tris/HCl, pH7.5, 15mM NaCl or in 10mM Tris/HCl, pH7.5, 1mM EDTA, pH8.0 (TE-buffer).

Working concentrations

The recommended working concentration is from 0.1 to 10 μ g/mL.
 10 μ g/mL (Removal of RNA from plasmid preparations: 1 hr, RT)
 100ng/mL (Preparation of "blunt ends" of double-stranded cDNA).

Stability

RNase A aggregates during lyophilisation and storage. It has a high affinity to glass surfaces, which has to be taken into consideration. At neutral pH (e.g. PBS pH7.4) and high concentrations (>10 mg/mL) the enzyme will precipitate. At 4°C (lyophilised) it is stable for several years (dry storage). The solution is stable for several years (if stored at -20°C) or several weeks (if stored at +4°C)

Specifications

Molecular weight:	approx. 13.7 kDa
CAS-Number:	[9001-99-4]
Activity:	min. 70 U/mg (Kunitz)
DNAases /Proteases:	not detectable
Storage:	- 20°C.

Unit definition One unit is defined as the amount of enzyme necessary to hydrolyse RNA to yield a velocity constant, $k = 1$, at 25°C and pH5.0 (also known as Kunitz-Unit).

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Elimination of DNase activity:

Product S5231 (RNase A (not certified DNase free)) may contain DNase activity. As RNase A is heat stable it is recommended to heat inactivate DNase activity before use.

Procedure:

10mg/mL RNase A in 0.01 sodium acetate (pH 5.2) have to be heated to 100°C for 15 minutes in a water bath. After 15 minutes, the RNase shall remain in the water bath while the water cools down to room temperature. pH can be adjusted by addition of 0.1 time the volume of 1M Tris-HCl (pH7.4). After aliquotation of the now DNase free RNase A solution, each aliquote should be stored a -20°C.

NOTE: RNase A precipitates if concentrated solutions are heated to 100°C at neutral pH!

Stability / Behaviour

RNase A aggregates while lyophilisation and storage.

The enzyme shows a strong affinity to glass surfaces, binding strongly to glass.

At neutral pH (for example PBS pH7.4) and at high concentrations (>10 mg/mL) the enzyme will precipitate.

Lyophilised and stored at a dry place at +4°C RNase A is stable for several years.

Stored as a solution at -20°C RNase A will be stable for several years.

Stored as a solution at +4°C RNase A will be stable for several weeks.