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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)

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. For example, the sequence of pG-pG-pC-pA-pG will be cleaved to give pG-pG-pCp and A-pG. The highest activity is exhibited with single stranded RNA. The pH-optimum is in the range of 7.0 to 7.5.
RNase A is a single chain polypeptide containing 4 disulfide bridges. In contrast to RNase B, it is not a glycoprotein.

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), ß-mercaptoethanol, heavy metals, vanadyl-ribonucleoside-complexes, RNAse-inhibitor from human placenta and competitively by DNA while the effect of denatured DNA is higher than by native nucleic acids, or by alkylation of His12 or His119, which are present in the active site of the enzyme. 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 (>300mM 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.

Specifications

Molecular weight: approx. 13.7 kDa
CAS-Number: [9001-99-4]
Activity: min. 70 U/mg (Kunitz)
Extinction coefficient: E(1%) = 7.1 (280 nm)
Isoelectric point: pI = 9.6.
Optimal temperature: 60°C (activity range of 15°C – 70°C)
Optimal pH: 7.6 (activity range of pH 6 – 10)
Activators of RNase A: potassium and sodium salts

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).

Stability / Behaviour

- RNAse A aggregates while lyophilisatioon 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 (>10mg/mL) the enzyme will precipitate.

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Storage: +2°C to +8°C at a dry place.
Stored as a solution at +2°C to +8°C RNAse A will be stable for several weeks.
Stored as a solution at -20°C RNAse A will be stable for several years.

Stock solutions

Recommended stock solutions are from 1 – 10mg/mL in 10mM Tris/HCl, pH7.4, 15mM NaCl or in 10mM Tris/HCl, pH7.4, 1mM EDTA, pH8.0 (TE-buffer).

Working concentrations

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

Elimination of DNAse activity (necessary for product S5231):

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 0.01M sodium acetate (pH5.2) must 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!

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