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## **Ribonuclease A from bovine pancreas (RNase A)**

Synonyms: Ribonuclease I, RNase A, Ribonuclease 3'-pyrimidinooligonucleotidohydrolase, Endoribonuclease I, Pancreatic ribonuclease

## For research use only

Cat No: YT9055 Size: 50 mg ( Lyophilized powder ) Store at −20°C Specific activity: ≥50 Kunitz units/mg protein Optimal PH: 7.6 (activity range of 6–10)

**Description:** RNase A is an endoribonuclease that attacks at the phosphate of a pyrimidine nucleotide. 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. RNase A is a single chain polypeptide containing disulfide bridges. Activators of RNase A include potassium and sodium salts.

Applications: Removal of RNA from recombinant protein preparations, Ribonuclease protection assays, Removal of non-hybridized regions of RNA:DNA hybrids, Mapping single-base mutations in DNA or RNA, Molecular weight marker. A major application for RNase A is the removal of RNA from preparations of plasmid DNA. For this application, DNase free RNase A is used at a final concentration of 10 µg /ml.

**Inhibitors:** Diethyl pyrocarbonate (DEPC), Guanidinium salts (4 M GuaSCN), β-mercaptoethanol, heavy metals, vanadyl-ribonucleoside complexes, RNase-inhibitor from human placenta, (denaturated) DNA (competitively)

**Reaction conditions:** Working concentration:  $1 - 100 \mu g/ml$  (depending on application). The enzyme is active under a wide range of reaction conditions. At low salt-concentrations (up to 100 mM NaCl), RNase A cleabes single- and double-stranded RNA and RNA in RNA:DNA hybrides. Under high salt concentrations (>300 mM NaCl) single-stranded RNA is cleaved only.

**Preparation Instructions:** Solutions prepared from powdered RNase A products can be made free of DNase by boiling. According to one literature method:

1. Prepare a 10 mg/mL stock solution in 10 mM sodium acetate buffer, pH 5.2.

2. Heat to 100°C for 15 minutes. Allow to cool to room temperature.

3. Adjust to pH 7.4 using 0.1 volume of 1 M Tris -HCl, pH 7.4 (i.e. add 500 µl 1M Tris-HCl, pH 7.4 to 5 ml of 10 mg/ml RNase stock solution).

4. Aliquot and store at  $-20^{\circ}$ C.

If RNase A is boiled at a neutral pH, precipitation will occur. When boiled at the lower pH, some precipitation may occur because of protein impurities that are present. Or Stock solutions are prepared at concentrations from 1 - 10 mg/ml in 10 mM Tris HCl (pH 7.5); 15 mM NaCl or in Tris HCl (pH7.5); 1 mM EDTA, pH 8 (TE buffer). The recommended working concentration is 10  $\mu$ g/ml(removal from RNA from plasmid preparations; 1 hr, RT) or 100 ng/ml (preparation of 'blunt ends' of double-stranded cDNA).

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