

## RIBONUCLEASE A

Product Number **R5125, R4875, R5503, R5000, R5250 and R5500**

Storage Temperature -20°C

CAS #: 9001-99-4

EC# 3.1.27.5

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

### Product Description

Molecular weight: 13,700 based upon the amino acid sequence<sup>1</sup>

Extinction coefficient: The E1% is 7.0 when measured at 280 nm<sup>2</sup>

Isoelectric point: The isoelectric point (PI) for this enzyme is 9.6<sup>3</sup>

Ribonuclease A (RNase A) is a single chain polypeptide containing 4 disulfide bridges. In contrast to RNase B it is not a glycoprotein.<sup>4</sup> RNase A can be inhibited by alkylation of the histidine-12 or histidine-119 which are present in the active site of the enzyme.<sup>5</sup> Activators of RNase A include potassium and sodium salts. The optimal temperature for activity is 60°C, although the enzyme does exhibit activity from 15-70°C. The pH optimum is 7.6, with an activity range of 6-10<sup>6</sup>. The highest activity is exhibited with single stranded RNA.<sup>7</sup> RNase A is a very stable enzyme and can withstand temperatures upto 100°C. At 100°C, RNase A is most stable between pH 2.0 and 4.5<sup>8</sup>.

RNase A is an endoribonuclease that attacks at the 3' 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.<sup>7</sup>

### Components

RNase A is isolated from bovine pancreas. A method of preparation is described in Crestfield, A.M. et.al.<sup>8</sup> This product is supplied as an essentially protease and salt-free lyophilized powder.

### Disclaimer/Precautions

RNase A is stable to both heat and detergents. In addition, it adsorbs strongly to glass. Scrupulous precautions are necessary to insure that residues of RNase A do not cause artifacts in processes requiring intact RNA.

### Preparation Instructions

When Sigma tests the activity of RNase A a stock solution is prepared in water at 1 mg/mL. Prior to usage, if the DNase impurity is a concern, one can use Sigma DNase free RNase that can be used directly (R6513 and R4642). Boiling of these products is not required prior to use.

RNase A can be made free of DNase by boiling. According to the following method described by Sambrook, J. Fritsch, E.F. and Maniatis, T.<sup>9</sup>, prepare a 10 mg/mL stock solution in 10 mM sodium acetate buffer (pH 5.2). Heat to 100°C for 15 minutes, allow to cool to room temperature, and then adjust to pH 7.4 using 0.1 volumes of 1 M TRIS/HCl pH 7.4. Aliquot and store at -20°C. If RNase A is boiled at a neutral pH, precipitation will occur. When boiled at a low pH, some precipitation may occur because of protein impurities that are present.

### Storage/Stability

RNase A is recommended to be stored in a freezer location (-20°C). Stock solutions stored in frozen aliquots are stable for at least 6 months.

### Procedure

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 ug/mL.<sup>10</sup>

Another use for RNase A is that can be used to test for complementarity between RNA:DNA hybrids.

### References

1. Smyth, D.G., Stein, W.H., Moore, S., J. Biol. Chem., 238, 227-234 (1963).
2. Pace, C.N., Vajdos, F., Fee, L., Grimsley, G., Gray, T., Protein Science, 4, 2411-2423 (1995).
3. Tanford, C. and Hauenstein, J. D., J. Am. Chem. Soc., 78, 5287-5291 (1956).
4. Plummer, T.H. and Hirs, C.H.W., J. Biol. Chem., 238, 1396-1397 (1963).
5. Heinrikson, R.L., Stein, W.H., Crestfield, A.M. and Moore, S., J. Biol. Chem., 240, 2921-2934 (1965).
6. Schomberg, D. and Salzman, M., Enzyme Handbook, Vol. 3, 1-3 under E.C. 3.1.27.5 (1990).

7. Burrell, M.M., Enzymes of Molecular Biology, Vol. 16, 263-270 (1993).
8. Crestfield, A.M., Stein, W.H., Moore, S., J. Biol. Chem., 238, 618-621 (1963).
9. Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning, A Laboratory Manual, 2<sup>nd</sup> ed., 1.51 (1989).
10. Sambrook, J., Fritsch, E.R, and Maniatis, T., Molecular Cloning, A Laboratory Manual, 2<sup>nd</sup> ed., B.17.

TMG 02/22/98

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.