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Product Information

Ribonuclease A from bovine pancreas

Catalog Number **R5500**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

CAS RN 9001-99-4
EC 3.1.27.5
Synonyms: RNase A; Pancreatic RNase;
Ribonuclease I; Endoribonuclease I;
Ribonuclease 3'-pyrimidinooligonucleotidohydrolase

Product Description

This product is isolated from New Zealand-sourced bovine pancreas. The method of preparation utilizes salt fractionation and chromatography. It is supplied as a lyophilized powder, essentially free of protease activity and salts.

Ribonuclease A (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.¹

RNase A is a single chain polypeptide containing 4 disulfide bridges. In contrast to RNase B, it is not a glycoprotein.² RNase A can be inhibited by alkylation of His¹² or His¹¹⁹, which are present in the active site of the enzyme.³ Activators of RNase A include potassium and sodium salts. Activity is observed in the pH range of 6-10 with optimal activity at pH 7.6.⁴ The optimal temperature for activity is 60 °C, although the enzyme does exhibit activity from 15–70 °C. RNase A is a very stable enzyme and can withstand temperatures up to 100 °C. At 100 °C, RNase A is most stable between pH 2.0 and 4.5.⁵

Molecular weight:⁶ 13.7 kDa (amino acid sequence)

Extinction coefficient:⁷ $E_{280}^{1\%} = 7.0$

Isoelectric point (pI):⁸ 9.6

Purity: $\geq 90\%$ RNase A (SDS-PAGE)

Activity: ~ 100 Kunitz units per mg protein

Protease activity: < 0.001 unit per mg solid

Disclaimer/Precautions

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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

Preparation Instructions

RNase A can be prepared free of DNase activity by boiling.⁹ Prepare a 10 mg/ml stock solution in 10 mM sodium acetate buffer, pH 5.2. Heat the solution to 100 °C for 15 minutes, cool to room temperature, and then adjust to pH 7.4 using 0.1 volume of 1 M Tris-HCl, pH 7.4. Aliquot and store at $-20\text{ }^{\circ}\text{C}$. If RNase A is boiled at a neutral pH, precipitation will occur. When boiled at a low pH, some precipitation may occur due to protein impurities that are present.

Alternatively, if the DNase impurity is a concern, one can use Sigma's DNase-free RNase preparations (Catalog Numbers R6513 and R4642). These can be used directly as boiling of these products is not required prior to use.

Storage/Stability

It is recommended to store the product at $-20\text{ }^{\circ}\text{C}$. This product is stable for at least 2 years when stored properly. 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 $\mu\text{g/ml}$.¹⁰

References

1. Burrell, M.M., *Enzymes of Molecular Biology*, Vol. 16, 263-270 (1993).
2. Plummer, T.H., and Hirs, C.H.W., *J. Biol. Chem.*, **238**, 1396-1397 (1963).
3. Heinrikson, R.L. *et al.*, *J. Biol. Chem.*, **240**, 2921-2934 (1965).
4. Schomberg, D., and Salzman, M., *Enzyme Handbook*, Vol. 3, 1-3 under E.C. 3.1.27.5 (1990).
5. Crestfield, A.M. *et al.*, *J. Biol. Chem.*, **238**, 618-621 (1963).
6. Smyth, D.G. *et al.*, *J. Biol. Chem.*, **238**, 227-234 (1963).
7. Pace, C.N. *et al.*, *Protein Science*, **4**, 2411-2423 (1995).
8. Tanford, C., and Hauenstein, J. D., *J. Am. Chem. Soc.*, **78**, 5287-5291 (1956).
9. Sambrook, J. *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd ed., 1.51 (1989).
10. Sambrook, J. *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd ed., B.17 (1989).

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