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# **Product Information**

# Ribonuclease A from bovine pancreas for molecular biology

Catalog Number **R6513**Storage Temperature –20 °C

CAS RN 9001-99-4

EC 3.1.27.5

Synonyms: Ribonuclease I; Pancreatic ribonuclease; Ribonuclease 3'-pyrimidinooligonucleotidohydrolase;

RNase A: Endoribonuclease I

## **Product Description**

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.<sup>1</sup>

RNase A is a single chain polypeptide containing 4 disulfide bridges. In contrast to RNase B, RNase A is not a glycoprotein.<sup>2</sup> RNase A can be inhibited by alkylation of His<sup>12</sup> or His<sup>119</sup>, which are present in the active site of the enzyme.<sup>3</sup> Activators of RNase A include potassium and sodium salts.

Molecular mass:4 13.7 kDa (amino acid sequence)

Extinction coefficient:  $E^{1\%} = 7.1$  (280 nm)

Isoelectric point:6 pl = 9.6

Optimal temperature: 60 °C (activity range of 15-70 °C)

Optimal pH:7 7.6 (activity range of 6-10)

Inhibitors: ribonuclease inhibitor

This chromatographically purified product is supplied as an essentially salt-free lyophilized powder.

Activity: ≥70 Kunitz<sup>8</sup> units/mg protein

A major application for RNase A is the removal of RNA from preparations of plasmid DNA. In this application, the presence of DNase activity as an impurity is a concern. The boiling-water bath method<sup>9</sup> used to eliminate contaminating DNase activity has proven unreliable. For this reason, Sigma-Aldrich developed a proprietary chromatographic preparation method for elimination of DNase activity.

In addition to protocols related to nucleic acid isolation,<sup>10</sup> this product has been used in studies of protein absorption on to solid surfaces<sup>11</sup> and in protein fractionation with charged membranes.<sup>12</sup>

#### **Precautions and Disclaimer**

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Note: RNase A is stable to both heat and detergents. In addition, it adsorbs strongly to glass. Scrupulous precautions are necessary to ensure that residual RNase A does not cause artifacts in processes that require intact RNA.

#### **Preparation Instructions**

When Sigma-Aldrich tests the activity of RNase A, a stock solution is prepared in water at 1 mg/mL.

Note: Boiling stock solutions of this RNase A product to inactivate residual DNase is not necessary, and may cause precipitation of RNase and possible loss of enzymatic activity. If an RNase A solution is heated at a neutral pH, precipitation will occur. When heated at a lower pH, some precipitation may occur because of protein impurities that are present.

### Storage/Stability

This product remains active for at least 3 years when stored properly at -20 °C.

RNase A is a very stable enzyme and solutions have been reported to withstand temperatures up to 100  $^{\circ}$ C. At 100  $^{\circ}$ C, an RNase A solution is most stable between pH 2.0 and 4.5.  $^{13}$ 

#### **Procedure**

For removal of RNA from preparations of plasmid DNA, DNase-free RNase A is used at a final concentration of  $10 \, \mu g/ml.^{14}$ 

#### References

- Sambrook, J., and Russell, D.W., Molecular Cloning, A Laboratory Manual (3<sup>rd</sup> ed). Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY), Volume 3, A4.39 (2001).
- Burrell, M.M., "RNase A (EC 3.1.27.5)", in Methods in Molecular Biology, Vol. 16: Enzymes of Molecular Biology (M.M. Burrell, ed.). Humana Press (Totowa, NJ), Ch. 13, pp. 263-270 (1993).
- 3. Plummer, T.H., Jr., and Hirs, C.H.W., *J. Biol. Chem.*, **238(4)**, 1396-1401 (1963).
- 4. Heinrikson, R.L. *et al.*, *J. Biol. Chem.*, **240(7)**, 2921-2934 (1965).
- Smyth, D.G. et al., J. Biol. Chem., 238(1), 227-234 (1963).
- Keller, P.J. et al., J. Biol. Chem., 233(2), 344-349 (1958).
- 7. Tanford, C., and Hauenstein, J. D., *J. Am. Chem.* Soc., **78(20)**, 5287-5291 (1956).

- 8. Schomberg, D., and Salzmann, M., *Enzyme Handbook*, Vol. 3, 1-3, under EC 3.1.27.5 (1990).
- 9. Kunitz, M., J. Biol. Chem., 164(2), 563-568 (1946).
- Till, B.J. et al., "Low-Cost DNA Extraction", in Low-Cost Methods for Molecular Characterization of Mutant Plants. Springer Cham / Springer Open, pp. 13-17 (2015).
- 11. Wei, Y. et al., Langmuir, **30(49)**, 14849-14858 (2014).
- 12. Sorci, M. et al., Biotechnol. Bioeng., **110(6)**, 1704-1713 (2013).
- 13. Crestfield, A.M. et al., J. Biol. Chem., **238(2)**, 618-621 (1963).
- Sambrook, J., and Russell, D.W., Molecular Cloning, A Laboratory Manual (3<sup>rd</sup> ed). Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY), Volume 1, 1.78-1.79 (2001).

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