

## Product Information

### Ribonuclease A Solution from bovine pancreas

Solution of 50% glycerol, for molecular biology

Catalog Number **R4642**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

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:<sup>4</sup> 13.7 kDa (amino acid sequence)

Extinction coefficient:<sup>5</sup>  $E^{1\%} = 7.1$  (280 nm)

Isoelectric point:<sup>6</sup>  $pI = 9.6$

Optimal temperature:  $60\text{ }^{\circ}\text{C}$  (activity range of  $15\text{-}70\text{ }^{\circ}\text{C}$ )

Optimal pH:<sup>7</sup> 7.6 (activity range of 6-10)

Inhibitors: ribonuclease inhibitor

The product is supplied in a solution containing 50% glycerol and 10 mM Trizma®-HCl, pH 8.0.

Activity:  $\geq 70$  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.

This product has been specifically used in studies of nuclear envelope isolation,<sup>10</sup> echinoderm embryos,<sup>11</sup> and molecular cytogenetics.<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

Note: Boiling stock solutions of Catalog No. R4642 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 2 years when stored properly at  $-20\text{ }^{\circ}\text{C}$ .

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

#### Procedure

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

## References

1. 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).
2. 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).
5. Smyth, D.G. *et al.*, *J. Biol. Chem.*, **238(1)**, 227-234 (1963).
6. 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).
10. Ori, A. *et al.*, "The Use of Targeted Proteomics to Determine the Stoichiometry of Large Macromolecular Assemblies", in *Methods in Cell Biology*, Vol. 122: *Nuclear Pore Complexes and Nucleocytoplasmic Transport – Methods* (V. Doye, ed.). Academic Press (San Diego, CA), Ch. 6, pp. 117-146 (2014).
11. Strickland, L. *et al.*, "Light Microscopy of Echinoderm Embryos", in *Methods in Cell Biology*, Vol. 74: *Development of Sea Urchins, Ascidians, and Other Invertebrate Deuterostomes: Experimental Approaches* (C.A. Ettensohn, G.A. Wray, and G.M. Wessel, eds). Elsevier Academic Press (San Diego, CA), Ch. 16, pp. 371-409 (2004).
12. Harrison, C.J. *et al.*, "Molecular Cytogenetics in Childhood Leukemia", in *Methods in Molecular Medicine*, Vol. 91: *Pediatric Hematology: Methods and Protocols* (N.J. Goulden and C.G. Steward, eds.). Humana Press (Totowa, NJ), Ch. 9, pp. 123-137 (2004).
13. Crestfield, A.M. *et al.*, *J. Biol. Chem.*, **238(2)**, 618-621 (1963).
14. 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).

Trizma is a registered trademark of Sigma-Aldrich Co. LLC.

RBG,MAM,KTA,GCY 08/19-2