

★ Storage Condition

Store Under -20°C .
 Keep dry. Warm to room temperature before opening.
 RNase A is properly lyophilized formulation maintaining adequate physical and chemical stability of the protein during shipping and long-term storage, even at ambient temperatures.

★ Shipping Condition

Ship with ice pack or dry ice.

★ Contents

RNase A (Pharmaceutical Grade), $\geq 3,000$ units/mg

★ Introduction

A major application for Ribonuclease A (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 used to eliminate contaminating DNase activity has proven unreliable. For this reason, GenDEPOT developed a proprietary chromatographic preparation method for elimination of DNase activity. 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. RNase A is a single chain polypeptide containing 4 disulfide bridges. In contrast to RNase B, it is not a glycoprotein. RNase A can be inhibited by alkylation of His¹² or His¹¹⁹, which are present in the active site of the enzyme.

★ Enzyme Information

Type	Native Enzyme
M. Wt	13.7 kDa (amino acid sequence)
Source	Bovine Pancreas
CAS No	9001-99-4
Activity	> 3,000 units per mg dry weight
Form	Lyophilized powder

★ Thermal Stability

RNase A is a very stable enzyme and solutions have been reported to withstand temperatures up to 100°C . At 100°C , an RNase A solution is most stable between pH 2.0 and 4.5.

★ Optimal Active Temperature

60°C (activity range $15-70^{\circ}\text{C}$).

★ Optimal Active pH

7.6 (activity range 6-10).

★ Inhibitor

Ribonuclease Inhibitor.

★ Unit Definition

One Unit hydrolyzes yeast RNA liberating soluble oligonucleotide causing an increase in absorbance at 260nm of 1.0 at 37°C , pH5.0.

★ Preparation of Stock Solution

Solutions prepared from powdered RNase A products can be made free of DNase by boiling. According to a literature method, 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 volume of 1 M Tris-HCl, pH 7.4. Aliquot and store -15°C ~ -25°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. Stock solutions stored in frozen aliquots remain active for at least 6 months.

★ RNA Removal using RNase A

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 0.2 mg/ml.