Ribonuclease A (RNase A)

DNase-free and Protease Free

Concentration: 20mg/ml, 20mg
Store at -20 ˚C
Cat. No.: MO5411

Description

Ribonuclease A (RNase A) is an endoribonuclease that specifically degrades single-standarded RNA at C and U residues. It cleaves the phosphodiester bond between the 5'-ribose of a nucleotide and the phosphate group attached to the 3'-ribose of an adjacent pyrimidine nucleotide. The resulting 2',3'-cyclic phosphate is hydrolyzed to the corresponding 3'-nucleoside phosphate.

Source

Bovine pancreas.

Molecular Weight

13.7 kDa monomer.

Definition of Activity Unit

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm when yeast RNA is hydrolyzed at 37^{O}C and pH 5.0.
Fifty units are approximately equivalent to 1 Kunitz unit.

Specific activity

≥ 5000 u/mg protein (≥100 Kunitz units/mg protein).

Concentration

Protein concentration is determined by measuring the absorbance at 278 nm using the molar absorption coefficient ε = 9800 $M^{-1}cm^{-1}$.

Storage Buffer

The enzyme is supplied in: 100 mM Tris-HCl (pH 8) and 50% (v/v) glycerol.

Applications

- Plasmid and genomic DNA preparation.
- Removal of RNA from recombinant protein preparations.
- Ribonuclease protection assays .
- Mapping single-base mutations in DNA or RNA.

Inhibitation and Inactivation

- Inhibitors: the most potent inhibitor is a ~ 50 kDa protein from the cytosol of mammalian cells, e.g., Ribonuclease Inhibitor (from human placenta)
  Phosphate, SDS, diethyl pyrocarbonate, 4M guanidinium thyocyanate plus 0.1M 2-mercaptoethanol and heavy metal ions.
- Inactivated by phenol/ chloroform extraction.

  other inhibitors:
  Uridine 2',3'-cyclic vanadate, 5'-diphosphoade-nosine 3'-phosphate and 5'-diphosphoadenosine 2'-

Note

- The working concentration of RNase A is 1-100µg/ml, depending on the application.
- The enzyme is active under a wide range of reaction conditions. At low salt concentrations (to 100mM NaCl), RNase A cleaves single-stranded and double-stranded RNA as well the RNA strand in RNA-DNA hybrids. However, at NaCl concentrations of 0.3M or higher, RNase A specifically cleaves single-stranded RNA.

Quality Control Assay Data

Endodeoxyribonuclease Assay

No detectable conversion of covalenty closed circular DNA was observed after incubation of 2.5µg of Ribonuclease A with 1µg of pBR322 DNA in 20 µl of buffer (10 mM Tris-HCl (pH 8 at 37^{o}C), 10mM $MgCl_2$)

Exodeoxyribonuclease Assay

No alteration of the banding pattern of DNA fragments was observed after incubation of 5µg of Ribonuclease A with 1µg of lambda

DNA/HindIII fragments in 20µl of buffer (10 mM Tris-HCl (pH 8 at 37^{o}C), 10mM $MgCl_2$) for 18 hours at 37^{o}C.

Protease Assay

No degradation of protease substrate was determined after incubation of 25 µg of Ribonuclease A with 200 µg of azocasein for 18 hours at 37^{o}C.

Functional Assay

Ribonuclease A was tested for RNA digestion in a plasmid DNA purification procedure.

Product Use Limitation

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

For Research Use Only

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