thermo scientific

PRODUCT INFORMATION

RNase A, DNase and Protease-free

Pub. No. MAN0012003
Rev. Date 09 January 2017 (Rev. B.00)

Lot: _ **Expiry Date: _**

Store at -20 °C

Components	#EN0531
RNase A, DNase and Protease-free, 10 mg/mL	10 mg

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For Research Use Only. Not for use in diagnostic procedures.

Description

RNase A is an endoribonuclease that specifically degrades single-stranded 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 (1, 2).

Applications

- Plasmid and genomic DNA preparation (3, 4).
- Removal of RNA from recombinant protein preparations.
- Ribonuclease protection assays. Used in conjunction with RNase T1 (3).
- Mapping single-base mutations in DNA or RNA (5, 6).

Source

Bovine pancreas.

Molecular Weight

13.7 kDa monomer.

Concentration

Protein concentration is determined by measuring the absorbance at 278 nm using molar absorption coefficient ε=9800 $M^{-1}cm^{-1}$ (7).

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 °C and pH 5.0. Fifty units are approximately equivalent to 1 Kunitz unit (8).

Specific activity

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

Storage Buffer

The enzyme is supplied in: 50 mM Tris-HCl (pH 7.4) and 50% (v/v) glycerol.

Inhibition and Inactivation

- Inhibitors: the most potent inhibitor is a mammalian ribonuclease inhibitor, e.g., Thermo Scientific RiboLock RNase Inhibitor (#EO0381).
  Other inhibitors:
  uridine 2',3'-cyclic vanadate, 5'-diphosphoadenosine 3'-phosphate and 5'-diphosphoadenosine 2'-phosphate (2), SDS, diethyl pyrocarbonate, 4 M guanidinium thiocyanate plus 0.1 M 2-mercaptoethanol and heavy metal ions.
- Not inactivated by heating, reliably removed by spin column or phenol/chloroform extraction.

Note

- Recommended concentration of RNase A is 1-100 µg/mL depending on the application.
- The enzyme is active under a wide range of reaction conditions. At low salt concentrations (0 to 100 mM NaCl), RNase A cleaves single-stranded and double-stranded RNA as well the RNA strand in RNA-DNA hybrids. However, at NaCl concentrations of 0.3 M or higher, RNase A specifically cleaves single-stranded RNA (9).

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with RNase A, DNase and Protease-free.

Labeled Oligonucleotide (LO) Assay

No detectable degradation after incubation of single-stranded or double-stranded radiolabeled oligonucleotides with RNase A.

Protease Assay

No detectable degradation of protease substrate after incubation of FTC-casein with RNase A.

Quality authorized by:  Jurgita Zilinskiene

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References

1. Blackburn, P., Moore S., Pancreatic ribonuclease, The Enzymes, V, (Boyer, P.D, ed.), Academic Press, New York, the third edition, vol. 15, 317-433, 1982.
2. Raines, R.T., Ribonuclease A, Chem.Rev., 98, 1045-1065, 1998.
3. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1.31-1.38, 2001.
4. Sharma, R.C., et al., A rapid procedure for isolation of RNA-free genomic DNA from mammalian cells, BioTechniques, 14, 176-178, 1993.
5. Myers R.M., et al., Detection of single base substitutions by ribonuclease cleavage at mismatches in RNA:DNA duplexes, Science 230, 1242-1246, 1985.
6. Winter E., et al., A method to detect and characterize point mutations in transcribed genes: Amplification and overexpression of the mutant c-Ki-ras allele in human tumor cells, Proc. Natl. Acad. Sci. USA, 82, 7575-7579, 1985.
7. Sela, M., Anfinsen, C.B., Some spectrophotometric and polarimetric experiments with ribonuclease, Biochim. Biophys. Acta, 24, 229-235, 1957.
8. Kunitz, M.A., A spectrophotometric method for the measurement of ribonuclease activity, J. Biol. Chem., 164, 563-568, 1946.
9. Ausubel, F.M., et al., Curent Protocols in Molecular Biology, vol1, John Willey & Sons, Inc., Brooklyn, New York, 3.13.1, 1994-2005.

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.

Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.