# thermo scientific

## PRODUCT INFORMATION RNase A, DNase and Protease-free

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## #EN0531

## Assembling Lot 0000000

Filling Lot 0000000

## Expiry Date MM.YYYY

Store at -25 °C to -15 °C

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

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#### 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  $\varepsilon$ =9800 M<sup>-1</sup>cm<sup>-1</sup> (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

 $\geq$ 5000 U/mg protein ( $\geq$ 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 (#E00381).

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 singlestranded 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

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- 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.
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- 8. Kunitz, M.A., A spectrophotometric method for the measurement of ribonuclease activity, J. Biol. Chem., 164, 563-568, 1946.
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