

CERTIFICATE OF ANALYSIS

# Ribonuclease T1

#EN0541 100,000u

Lot: Quality guaranteed:

Concentration: 1000u/ $\mu$ l

Store at  $-20^{\circ}\text{C}$

2

In total 1 vial.

## Description

Ribonuclease T1 (RNase T1) is an endoribonuclease that specifically degrades single-stranded RNA at G residues. It cleaves the phosphodiester bond between 3'-guanylic residues and the 5'-OH residues of adjacent nucleotides with the formation of the corresponding intermediate 2', 3'-cyclic phosphates (1). The reaction products are 3'-GMP and oligonucleotides with a terminal 3'-GMP.

## Source

*E.coli* cells carrying a cloned *mtA* gene of *Aspergillus oryzae*.

## Unit Definition

One unit of the enzyme causes an increase in absorbance of 1.0 at 260nm in 15 minutes when yeast RNA is hydrolyzed at  $37^{\circ}\text{C}$  and pH 7.5.

## Activity Assay

50mM Tris-HCl (pH 7.5), 2mM EDTA, 3mg/ml yeast RNA.

## Storage Buffer

50mM Tris-HCl (pH 7.4) and 50% glycerol.

## Applications

- RNA sequencing (1).
- Ribonuclease protection assays. Used in conjunction with RNase A (2).
- Removal of RNA from DNA preparations.
- Determination of the level of RNA transcripts synthesized *in vitro* from DNA templates containing a "G-less cassette" (3).

## Inactivation

By phenol/chloroform extraction.

## QUALITY CONTROL ASSAY DATA

### **Endodeoxyribonuclease Assay**

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 1000 units of Ribonuclease T1 with 1µg of pBR322 DNA in 50µl of buffer (10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl<sub>2</sub>) for 18 hours at 37°C.

### **Exodeoxyribonuclease Assay**

No alteration of the banding pattern of DNA fragments was observed after incubation of 1000 units of Ribonuclease T1 with 1µg of lambda DNA/HindIII fragments in 50µl of buffer (10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl<sub>2</sub>) for 18 hours at 37°C.

### **Labeled Oligonucleotide (LO) Assay**

No detectable degradation of a single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 1000 units of Ribonuclease T1 in 10mM Tris-HCl (pH 7.5 at 37°C) buffer, containing 10mM MgCl<sub>2</sub> for 18 hours at 37°C.

### **Protease Assay**

No degradation of protease substrate was determined after incubation of 10000 units of Ribonuclease T1 with 200µg of azocasein for 18 hours at 37°C.

### **Functional Assay**

Ribonuclease T1 in conjunction with Ribonuclease A (DNase and protease free) was tested for RNA digestion in the plasmid DNA purification procedure.

Quality authorized by:



Jurgita Zilinskiene

## **References**

1. Takahashi K., Moore S., Ribonuclease T1, The Enzymes, V, (Boyer, P.D, ed.), Academic Press, New York, the third edition, vol. 15, 435-468, 1982.
2. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 7.63-7.74, 2001.
3. Sawadogo, M., Roeder, R.G., Factors involved in specific transcription by human RNA polymerase II: Analysis by a rapid and quantitative *in vitro* assay, Proc. Natl. Acad. Sci. USA, 82, 4394-4398, 1985.

## **Related Products**

- Ribonuclease A #EN0531
- RNase A/T1 Mix #EN0551
- Proteinase K #E00491  
#E00492
- Genomic DNA Purification Kit #K0512
- Water, nuclease-free #R0581  
#R0582
- DEPC-treated Water #R0601  
#R0603

## **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.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.