

CERTIFICATE OF ANALYSIS RNase A/T1 Mix

#EN05511 mlLot:Expiry Date:

Concentration: 2 mg/ml of RNase A and 5000 u/ml of RNase T1

Store at -20°C

In total 1 vial.

Description

RNase A/T1 Mix combines the RNA degradation activity of both RNase A and RNase T1. The RNase A specifically hydrolyzes RNA at C and U residues;

RNase T1 specifically hydrolyzes RNA at G residues (1).

Applications

- Removal of RNA from DNA preparations (2).
- Removal of RNA from recombinant protein preparations.
- Ribonuclease protection assays (1, 2).

Source

RNase A: Bovine pancreas. RNase T1: *E.coli* cells with a cloned *mtA* gene of *Aspergillus oryzae.*

Unit Definition for RNase A

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 (3).

Unit Definition for RNase T1

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm in 15 min when yeast RNA is hydrolyzed at 37°C and pH 7.5.



Storage Buffer

The enzymes are supplied in: 50 mM Tris-HCl (pH 7.4), 50% (v/v) glycerol.

Inactivation

Not inactivated by heating, reliably removed by spin column or phenol/chloroform extraction.

Recommendations for Use

RNase digestion mixture for RNase protection assay (1): 10 mM Tris-HCl (pH 7.5), 300 mM NaCl, 5 mM EDTA (pH 7.5), 20 µl of RNase A/T1 Mix per 1 ml of reaction mixture.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 2 μ l of RNase A/T1 Mix with 1 μ g of pUC19 DNA in 20 μ l of buffer (25 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 5 mM NaCl) for 18 hours at 37°C.

Protease Assay

No degradation of protease substrate was determined after incubation of 10 μ l RNase A/T1 Mix with 200 μ g of azocasein 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 2 μ l of RNase A/T1 Mix in buffer, (25 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 5 mM NaCl) for 18 hours at 37°C.

Quality authorized by:



Jurgita Zilinskiene

References

- Ausubel, F.M., et al., ed., Current Protocols in Molecular Biology, vol.1, John Wiley & Sons, Inc., Brooklyn, New York, 3.13.1-3.13.3, 1994-2004.
- 2. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
- Kunitz, M.A., A spectrophotometric method for measurement of ribonuclease activity, J. Biol. Chem., 164, 563-568, 1946.

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 <u>www.fermentas.com</u> for Material Safety Data Sheet of the product.