Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Ribonuclease I, *E.coli*

#EN0601 1000u

Lot: **Quality guaranteed:**

Concentration: 10u/µl

Store at –20°C

In total 1 vial.

ISO 9001 ISO 14001
www.fermentas.com

Description

Ribonuclease I (RNase I), an endoribonuclease, specifically degrades single-stranded RNA at all dinucleotide bonds to nucleoside 3'-monophosphates via nucleoside 2'-, 3'-cyclic monophosphate intermediates (1).
The enzyme does not require any metal ions for activity.

Source

E.coli cells carrying the cloned *rna* gene.

Unit Definition

One unit of the enzyme catalyzes degradation of 100ng of *E.coli* ribosomal RNA per second into acid-soluble nucleotides at 37°C.

Activity Assay

50mM Tris-acetate (pH 8.0), 100mM NaCl,
0.1mM EDTA, 0.01% Triton X-100,
40µg/ml *E.coli* ribosomal [^{3}H]-RNA.

Storage Buffer

50mM Tris-HCl (pH 8.0), 100mM NaCl,
0.01% Triton X-100 and 50% glycerol.

Applications

- Removal of RNA from DNA solutions (2).
- Ribonuclease protection assays (3).

Inactivation

By heating at 100°C for 30min, phenol/chloroform extraction or with SDS-Proteinase K.

Note

- RNase I does not degrade DNA, but binds to it.
- Mammalian ribonuclease inhibitors have no effect on RNase I.
- RNase I amino acid sequence is typical for the RNase T2 family.
- Non-ionic detergents (e.g. Triton X-100) do not inhibit RNase I, and may even slightly stimulate its activity and stabilize it against heat inactivation. Triton X-100 or BSA (at 0.1mg/ml) may protect RNase I from sticking to glass vessels when working with highly dilute solutions.
- Polyamines stimulate the activity of RNase I.
- RNase I is less stable against heat inactivation than RNase A. Low concentrations of ionic detergents (e.g. 0.1% SDS) act as potent and irreversible inhibitors.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 80 units of Ribonuclease I with 1µg of pBR322 DNA in 50µl of buffer (50mM Tris-acetate (pH 8.0), 100mM NaCl, 0.1mM EDTA, 10mM $MgCl_2$) for 4 hours at 37°C.

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 80 units of Ribonuclease I with 1µg of sonicated *E.coli* [^{3}H]-DNA in 50µl of buffer (50mM Tris-acetate (pH 8.0), 100mM NaCl, 0.1mM EDTA, 10mM $MgCl_2$) for 4 hours at 37°C.

Quality authorized by: Jurgita Zilinskiene

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Reference

1. Shen, V., Schlessinger, D., RNase I *of Escherichia coli*, The Enzymes (Boyer, P.D., ed), Academic Press Inc., New York, vol. 15B, 503-506, 1982.
2. Zhu, L., Deutscher, M.P., The *Escherichia coli rna* gene encoding RNase I: sequence and unusual promoter structure, Gene, 119, 101-106, 1992.
3. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.

Related Product

- BSA, molecular biology grade #B14
- Water, nuclease-free #R0581 #R0582

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 for Material Safety Data Sheet of the product.

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