

CERTIFICATE OF ANALYSIS

**RiboLock™
RNase Inhibitor**

#E00384 24 x 2500 u

Lot: **Expiry Date:**

Concentration: 40 u/μl

Store at -20°C

In total 24 vials.

Description

RiboLock™ RNase Inhibitor inhibits the activity of RNases A, B, C by binding them in a noncompetitive mode at a 1:1 ratio. It does not inhibit the following RNases: I, T1, T2, H, U1, U2 and CL3.

Source

E.coli cells with a cloned gene encoding a mammalian ribonuclease inhibitor.

Molecular Weight

49.6 kDa monomer.

Definition of Activity Unit

One unit of RiboLock™ RNase Inhibitor inhibits the activity of 5 ng of RNase A by 50%.

Inhibitor activity is assayed in the following mixture:
100 mM Tris-HCl (pH 7.5), 1.2 mM EDTA,
0.1 mg/ml BSA, 100 ng/ml RNase A,
0.1 mg/ml *E.coli* [³H]-RNA, 50 mg/ml yeast RNA,
8 mM DTT.

Storage Buffer

The protein is supplied in: 20 mM HEPES-NaOH (pH 7.5), 50 mM NaCl, 8 mM DTT, 0.5 mM ELUGENT Detergent and 50% (v/v) glycerol.

Applications

- Inhibition of RNA degradation in the following procedures:
 - *in vitro* transcription (1);
 - cDNA synthesis (2);
 - *in vitro* translation (3);
 - isolation of mammalian cell fractions that contain mRNA-protein complexes (3);
- Separation and identification of specific ribonuclease activities (4);
- Tumor suppression studies (5).

Note

- DTT is not necessary for Ribolock™ RNase Inhibitor activity. It is only required to ensure stability of the inhibitor during long-term storage.
- Working concentration – 1 unit per µl of a reaction mixture.

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QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 200 units of RiboLock™ RNase Inhibitor with 1 µg of pBR322 DNA in 50 µl of buffer (40 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 10 mM DTT, 10 mM NaCl and 2 mM spermidine) for 4 hours at 37°C.

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into the trichloroacetic acid-soluble fraction after incubation of 200 units of RiboLock™ RNase Inhibitor with 1 µg of sonicated *E.coli* [³H]-DNA in 50 µl of buffer (40 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 10 mM DTT, 10 mM NaCl and 2 mM spermidine) for 4 hours at 37°C.

Latent Ribonuclease Assay

0% of the total radioactivity was released into the trichloroacetic acid-soluble fraction after incubation of 200 units of RiboLock™ RNase Inhibitor (heated for 15 min at 70°C) with 1 µg of [³H]-RNA in 50 µl of buffer (40 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 10 mM DTT, 10 mM NaCl and 2 mM spermidine) for 4 hours at 37°C.

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Ribonuclease Assay

0% of the total radioactivity was released into the trichloroacetic acid-soluble fraction after incubation of 200 units of RiboLock™ RNase Inhibitor with 1 µg of [³H]-RNA in 50 µl of buffer (40 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 10 mM DTT, 10 mM NaCl and 2 mM spermidine) for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 200 units of RiboLock™ RNase Inhibitor for 4 hours at 37°C.

Quality authorized by:



Jurgita Zilinskiene

References

1. Nielsen, D.A., Shapiro, D.J., Preparation of capped RNA transcripts using T7 RNA polymerase, *Nucleic Acids Res.*, 14, 5936, 1986.
2. Martynoff, G., et al., Synthesis of a full length DNA complementary to thyroglobulin 33S messenger RNA, *Biochem. Biophys. Res. Commun.*, 93, 645-653, 1980.
3. Scheele, G., Blackburn, P., Role of mammalian RNase inhibitor in cell-free protein synthesis, *Proc. Natl. Acad. Sci. USA*, 76, 1898-1902, 1979.
4. Eichler, D.C., et al., Effect of human placental ribonuclease inhibitor in cell-free ribosomal RNA synthesis, *Biochem. Biophys. Res. Commun.*, 101, 396-403, 1981.
5. Polakowski, I.J., et al., A ribonuclease inhibitor expresses anti-angiogenic properties and leads to reduced tumor growth in mice, *Amer. J. Pathol.*, 143, 507-517, 1993.

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.

Related Products

- SP6 RNA Polymerase #EP0131
- T3 RNA Polymerase #EP0101, #EP0102, #EP0103
- T7 RNA Polymerase #EP0111, #EP0112, #EP0113
- T7 Transcription Kit #K0411, #K0412
- M-MuLV Reverse Transcriptase #EP0351, #EP0352
- RevertAid™ M-MuLV Reverse Transcriptase (not available in the USA) #EP0441, #EP0442
- RevertAid™ H Minus M-MuLV Reverse Transcriptase (not available in the USA) #EP0451, #EP0452
- First Strand cDNA Synthesis Kit #K1611, #K1612
- RevertAid™ First Strand cDNA Synthesis Kit (not available in the USA) #K1621, #K1622
- RevertAid™ H Minus First Strand cDNA Synthesis Kit (not available in the USA) #K1631, #K1632
- T4 RNA Ligase #EL0021
- DTT #R0861, #R0862
- DEPC-treated Water #R0601, #R0603