

**CERTIFICATE OF ANALYSIS**

**RiboLock™  
RNase Inhibitor**

**#E00381**      2500 u

**Lot:**                      **Expiry Date:**

Concentration:      40 u/μl

**Store at -20°C**

In total 1 vial.

**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* [<sup>3</sup>H]-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 MgCl<sub>2</sub>, 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* [<sup>3</sup>H]-DNA in 50 µl of buffer (40 mM Tris-HCl (pH 7.9), 6 mM MgCl<sub>2</sub>, 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 [<sup>3</sup>H]-RNA in 50 µl of buffer (40 mM Tris-HCl (pH 7.9), 6 mM MgCl<sub>2</sub>, 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 [<sup>3</sup>H]-RNA in 50 µl of buffer (40 mM Tris-HCl (pH 7.9), 6 mM MgCl<sub>2</sub>, 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](http://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