

## RNase Inhibitor, Murine



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M0314S 027170119011

# M0314S



**3,000 units 40,000 U/ml Lot: 0271701**

**Recombinant Store at -20°C Exp: 1/19**

**Description:** RNase Inhibitor, Murine is a 50 kDa recombinant protein. The inhibitor specifically inhibits RNases A, B and C. It inhibits RNases by binding noncovalently in a 1:1 ratio with high affinity. It is not effective against RNase 1, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. In addition, no inhibition of polymerase activity is observed when RNase Inhibitor is used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

Recombinant murine RNase inhibitor does not contain the pair of cysteines identified in the human version that is very sensitive to oxidation, which causes inactivation of the inhibitor (1). As a result, RNase Inhibitor, Murine has significantly improved resistance to oxidation compared to the human/porcine RNase inhibitors, and is stable at low DTT concentrations (< 1 mM). This makes it ideal for reactions where high concentration DTT is adverse to the reaction (eg. Real-time RT-PCR).

**Source:** An *E. coli* strain that carries the Ribonuclease Inhibitor gene from mouse.

### Applications:

- RT-PCR
- cDNA synthesis
- *In vitro* transcription/translation
- Enzymatic RNA labeling reaction
- Other applications where the integrity of RNA is important

Supplied in: 50 mM KCl, 20 mM HEPES-KOH (pH 7.6), 8 mM DTT and 50% glycerol.

**Unit Definition:** One unit is defined as the amount of RNase Inhibitor, Murine required to inhibit the activity of 5 ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2, 3'-cyclic monophosphate by RNase A.

### Quality Control Assays

**Endonuclease Activity:** Incubation of a 10 µl reaction containing 40 units of RNase Inhibitor, Murine with 300 ng supercoiled plasmid for 4 hours at 37°C produced < 10% nicked molecules as determined by gel electrophoresis.

**RNase Assay:** Incubation of a 10 µl reaction containing 40 units of RNase Inhibitor, Murine with 40 ng of RNA transcript for 4 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Latent RNase Assay:** Heating the RNase Inhibitor, Murine for 20 minutes at 65°C, followed by incubation of a 10 µl reaction containing 40 units of RNase Inhibitor with 40 ng of RNA transcript for 4 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Exonuclease Assay:** Incubation of a 50 µl reaction containing 200 units of RNase Inhibitor, Murine with 1 µg of a mixture of single and double stranded [<sup>3</sup>H] *E. coli* DNA (10<sup>5</sup> cpm/µg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

**Notes On Use:** Since ribonucleases typically retain activity under denaturing conditions, care must be taken to avoid denaturing RNase Inhibitor molecules which have complexed with a ribonuclease. To prevent the release of active ribonuclease, temperatures greater than 50°C and high concentrations of urea or other denaturing agents should be avoided.

The recommended concentration of RNase Inhibitor in a reaction is 1 unit/µl. During assembly of a reaction, RNase Inhibitor should be added before other components that are a possible source of RNase contamination (i.e. enzymes, plasmid from a mini prep.)

### Reference:

1. Kim, B.M. et al. (1999) *Protein Science*, 8, 430-434.

CERTIFICATE OF ANALYSIS

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