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## RNase Inhibitor (cloned)

Product No. RNI2500

RNI10000

PureBiotech, LLC, **RNase Inhibitor** is a recombinant 50 kD protein purified from an overexpressing strain of *E. coli*. This enzyme inhibits RNase by tightly binding ( $Ka = 10^{14}$ ) RNase A, B or C-type enzymes in a 1:1 molar ratio. The binding reaction requires a minimum of 1 mM DTT for maximum effectiveness and is active over a broad pH range. RNase Inhibitor is guaranteed nuclease-free and has been demonstrated not to inhibit nucleic acid modifying enzymes commonly used in molecular biology applications. For example, it does not inhibit AMV or MMLV reverse transcriptase, *Taq* DNA polymerases, phage RNA polymerases, RNase 1, RNase H, RNase T1, or S1 Nuclease, or RNase from *Aspergillus*. Use of RNase Inhibitor increases yields in cDNA synthesis reactions, *in vitro* transcription, *in vitro* translation, and other reactions that utilize RNA substrates or produce RNA.

Concentration: 20,000 units/ml (2500 units/vial)

**Unit Definition:** One unit is the amount of enzyme required to inhibit the activity of 5 ng of ribonuclease A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2',3'-cyclic monophosphate by ribonuclease A, where 200 units of RNase Inhibitor produces 50% inhibition of ribonuclease A.

Storage Buffer: 20 mM Hepes-KOH (pH 7.6), 50 mM KCl, 8 mM dithiothreitol, 50% (v/v) glycerol.

**Storage Conditions:** -20°C. DO NOT STORE IN A FROST-FREE FREEZER. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes. For long term storage (> 1 month) storage at -80°C is recommended.

**Physical Purity:** 90% by SDS PAGE stained with Coomassie Blue.

**Ribonuclease Assay**: 1 µg of RNA is incubated with 200 units RNase Inhibitor for 60 minutes at 37°C. The RNA is electrophoresed on an agarose gel and visualized by ethidium bromide staining. No ribonuclease activity is observed. When this assay is carried out with 200 units pre-heated RNase Inhibitor for 60 minutes at 37°C no latent ribonuclease activity is observed.

Less than 3% release of label form 50 ng [3H]RNA when 200 units of either RNase Inhibitor or pre-heated RNase Inhibitor are incubated with RNA for 1 hour at 37°C.

**DNase Assay:** Less than 3% release of label form 50 ng [3H]DNA when 200 units of either RNase Inhibitor or pre-heated RNase Inhibitor are incubated with DNA for 1 hour at 37°C.

**Endonuclease Activity:** Not detected. Incubation of 200 units of RNase Inhibitor with super-coiled plasmid DNA produced no nicked molecules after one hour at 37°C as determined by agarose gel electrophoresis.

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