PRODUCT INSERT

PUREGENE GENETIX BRAND

Riboblock RNase Inhibitor

Store at -20°C

Cat. No.	Pack Size
PGM038	3000U

Concentration: 40 U/µl

Description
Puregene Riboblock RNase Inhibitor inhibits the activity of RNases A, B and C by binding them in a noncompetitive mode at a 1:1 ratio. It does not inhibit eukaryotic RNases: T1, T2, U1, U2, CL3 as well as prokaryotic RNases I and H.

Applications
• Inhibition of RNA degradation in the following:
– in vitro transcription
– cDNA synthesis
– in vitro translation
– isolation of mammalian cell fractions that contain mRNA-protein complex
– RNA amplification
• RNA purification and storage.
• Separation and identification of specific ribonuclease activities
• Studies of tumor suppression

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 the Riboblock 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 [3H]-RNA, 50 mg/ml yeast RNA, 8 mM DTT.

Storage (Dilution) Buffer
The protein is supplied in: 20 mM HEPES-NaOH (pH 7.5), 50 mM NaCl, 8 mM DTT, 0.03% (v/v) ELUGENT Detergent and 50% (v/v) glycerol.

Inhibition and Inactivation
• Inhibitors: common denaturants (SDS, urea and all oxidizing reagents (p-chloromercuribenzoate, dissolved oxygen, ions in their higher oxidation states) strongly inhibit RiboLock RNase Inhibitor and release the RNase bound.

• Inactivated by heating at 75°C for 10 min. Residual activity detectable after 10 min heating at 70°C.

Note
• DTT provided in the Storage Buffer ensures stability during long term storage, but is not necessary for inhibitor activity.

• Recommended concentration – 1 u/µl of a reaction mixture.

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 200 units of Riboblock RNase Inhibitor with 1 µg of pUC19 DNA for 4 hours at 37°C.

Latent Ribonuclease Assay

No latent RNase activity was detected after incubation of 200 units of Riboblock RNase Inhibitor (heated for 15 min at 70°C) with 1 µg of [3H]-RNA for 4 hours at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 200 units of Riboblock RNase Inhibitor with 1 µg of [3H]-RNA for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No degradation of single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 80 units of Riboblock RNase Inhibitor for 4 hours at 37°C.