RNase Inhibitor, Human Placenta

1-800-632-7799
info@neb.com
www.neb.com

M0307S 023130115011

M0307S

2,000 units **40,000 U/ml** **Lot: 0231301**

RECOMBINANT **Store at –20°C** **Exp: 1/15**

Description: RNase Inhibitor, Human Placenta is a recombinant human placental protein which specifically inhibits ribonucleases (RNases) A, B and C (1). 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, Human Placenta is used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

The 50 kDa protein inhibits RNases by binding noncovalently in a 1:1 ratio with an association constant greater than 10^{14} (2).

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

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

Applications:

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

Unit Definition: One unit is defined as the amount of RNase Inhibitor, Human Placenta 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, Human Placenta with 300 ng supercoiled plasmid for 4 hours at 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, Human Placenta 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, Human Placenta 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, Human Placenta with 1 µg of a mixture of single and double stranded [^{3}H] *E. coli* DNA (10^5 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 possible sources of RNase contamination (i.e. enzymes, plasmid from a mini prep.)

CERTIFICATE OF ANALYSIS

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References:

1. Blackburn, P. and Moore, S. (1982) *Pancreatic Ribonucleases,* In: *The Enzymes,* Vol XV, Part B Academic Press, N.Y.
2. Blackburn, P., Wilson, G. and Moore, S. (1977) *J. Biol. Chem.* 252, 5904.