

RNase Inhibitor (Cloned)

Catalog Number AM2682, AM2684

Pub. No. 4393874 Rev. B

Contents	Quantity	Storage conditions
RNase-Inhibitor (Cloned), 40 U/μL	Cat. no. AM2692: 2,500 Units	Store at -20°C. <i>Do not store in a frost-free freezer.</i>
	Cat. no. AM2694: 10,000 Units	

 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Product description

RNase Inhibitor, a recombinant human protein produced in *E. coli* (Blackburn, 1979), is a potent inhibitor of neutral pancreatic ribonucleases, including RNases A, B, and C. The mode of inhibition is noncompetitive; the inhibitor tightly binds RNases in a 1:1 ratio. The enzyme has been shown to inactivate a variety of RNases that are present in many tissues and cell types. RNase Inhibitor does not inhibit RNase T1, RNase 1, RNase H, S1 Nuclease, or RNase from *Aspergillus*.

Source: Recombinant *E. coli* strain.

Unit (U) definition: One unit is the amount of protein required to inhibit the activity of 5 ng of RNase A by 50%. Unit assay conditions: 100 mM Tris-acetate (pH 6.5), 1 mM EDTA, 1 mM cyclic 2',3'-CMP, and RNase inhibitor. Addition of RNase A initiates the reaction. RNase inhibitor activity is measured by the inhibition of hydrolysis of cyclic 2',3'-CMP by RNase A.

Storage buffer (not included): 20 mM HEPES-KOH (pH 7.6). 50 mM KCl, 5 mM DTT and 50% (v/v) glycerol. If opened frequently during storage, we recommend adding DTT to maintain the optimal 5 mM level.

Using RNase Inhibitor

Addition of RNase Inhibitor has been shown to be useful whenever the integrity of RNA must be maintained, such as in the preparation of cDNA by reverse transcription (deMartynoff, et. al., 1980), *in vitro* RNA transcription (Melton, et. al.1984), and *in vitro* protein synthesis (Scheele and Blackburn, 1979).

RNase Inhibitor requires a minimum of 1 mM DTT to maintain activity and requires a pH of 5–8, with maximal activity between

pH 7 and 8. Since the mode of inhibition is the formation of a 1:1 complex with RNases, avoid denaturation or oxidation of RNase Inhibitor, which would result in the release of active RNase. The half-life of RNase Inhibitor:RNase A binding is approximately 8 hr.

Guidelines for RNA transcription, protein translation, and cDNA synthesis

Add RNase Inhibitor to transcription, translation, and cDNA synthesis reactions at a final concentration of 1 U/μL. RNase Inhibitor requires a minimum of 1 mM DTT and is active over a broad pH range. Avoid denaturation of RNase Inhibitor by SDS, urea, etc., which could result in a release of active RNases.

Limited product warranty

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References

- Blackburn, P. 1979. Ribonuclease inhibitor from human placenta: rapid purification and assay. *J. Biol. Chem.* 254: 12484–12487.
- deMartynoff, G., Pays, E. and Vassart, G. 1980. Synthesis of a full length DNA complementary to thyroglobulin 33 S messenger RNA. *B.B.R.C.* 93: 645–653.
- Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K. and Green, M.R. 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucl. Acids Res.* 12, 70335–70356.
- Scheele, G. and Blackburn, P. 1979. Role of mammalian RNase inhibitor in cell-free protein synthesis. *P.N.A.S. USA* 76: 4898–4902.

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