

# RNasin® Ribonuclease Inhibitor Part II: A Tale of Two Proteins



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## ABSTRACT

In this article we present data that compares RNasin® Ribonuclease Inhibitor to SUPERase•In™, an RNase inhibitor recently introduced by Ambion. The two RNase inhibitors are compared for their ability to protect RNA from degradation under a variety of different reaction conditions. In addition, we tested both inhibitors for their ability to protect RNA during *in vitro* translation and transcription.

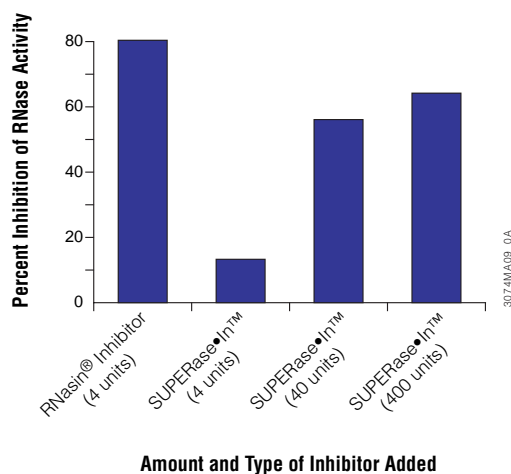
## INTRODUCTION

Many applications in molecular biology including cDNA synthesis, RT-PCR<sup>(a)</sup> and *in vitro* transcription and translation require the use of intact RNA, and investigators often rely on ribonuclease inhibitors to minimize the degradation of RNA samples by RNase. Since contaminating enzyme activities and nucleic acids can interfere with these applications, the RNase inhibitor chosen should have several important features. The inhibitor should effectively protect RNA during laboratory procedures and in dilute solutions. For applications such as RT-PCR, recombinant RNase inhibitors are advantageous because they are essentially free of contaminating eukaryotic DNA. This article compares the performance of two commercially available RNase inhibitors.

Just four units\* of Promega's RNasin® Ribonuclease Inhibitor provides greater inhibition than 400 units of SUPERase•In™ (Figure 1).

## COMPARISON OF RNASIN® RIBONUCLEASE INHIBITOR AND SUPERASE•IN™

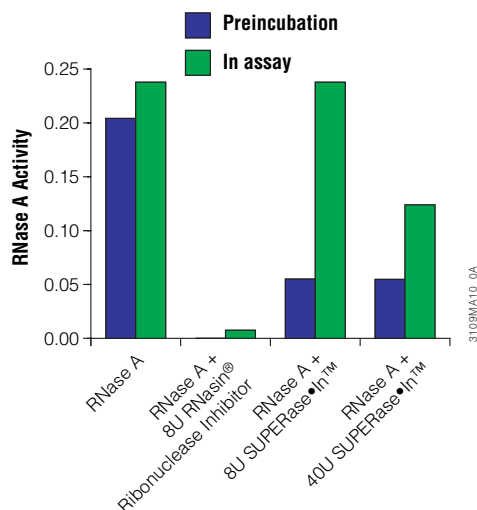
Comparison of RNasin® Ribonuclease Inhibitor<sup>(b,c)</sup> and SUPERase•In™ using the total yeast RNA assay (1) indicates that both inhibitors offer some protection of RNA from degradation, but SUPERase•In™ appears to be less effective than RNasin® Ribonuclease Inhibitor. Just four units\* of Promega's RNasin® Ribonuclease Inhibitor provides greater inhibition than 400 units of SUPERase•In™ (Figure 1).



**Figure 1. Comparison of RNasin® Ribonuclease Inhibitor and SUPERase•In™ inhibition of RNase A activity in the total yeast RNA assay.** Total yeast RNA was incubated in the presence of 5ng RNase A for 15 minutes at 37°C in 0.5ml of reaction mix containing 50mM MOPS and 5mM MgCl<sub>2</sub> (pH 6.5). After incubation, 0.5ml 10% TCA was added to stop the reaction and to precipitate the large RNA molecules. An OD<sub>280</sub> measurement was taken of the TCA-soluble material.

Explanations of these results include the possibility that SUPERase•In™ binds RNase slowly or that it needs to be preincubated with the RNase at relatively high concentrations before it demonstrates substantial RNase inhibition. We repeated the total yeast RNA assay comparing preincubation of the inhibitors with RNase (“preincubated”) to direct addition of inhibitor to the assay (“in assay”, Figure 2). As expected, RNasin® Ribonuclease Inhibitor strongly inhibits RNase activity in both the “preincubation” and the “in assay” tests (Figure 2). SUPERase•In™ shows substantially more inhibition of RNase in the preincubation test than in the “in assay” test, but even under conditions of preincubation, 40 units of SUPERase•In™ fail to protect the RNA as well as 8 units of RNasin® Ribonuclease Inhibitor.

\*Unit is defined as the amount of ribonuclease inhibitor required to inhibit by 50% the activity of 5ng RNase A based on the inhibition of cytidine 2' 3' cyclic monophosphate hydrolysis (2,3).

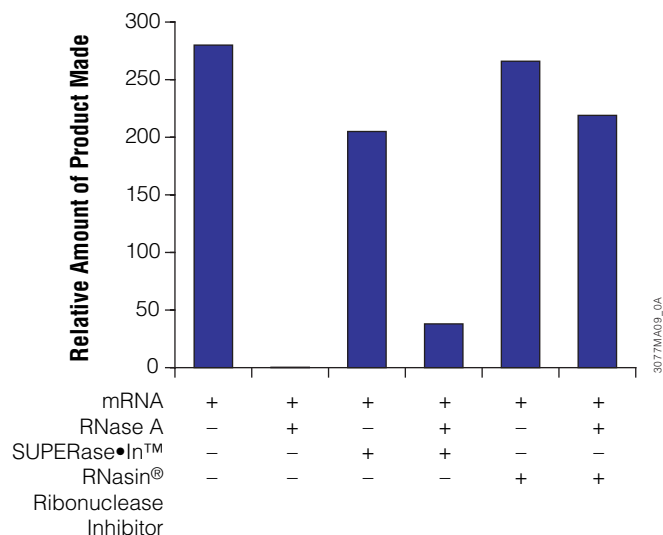


**Figure 2. Comparison of RNasin® Ribonuclease Inhibitor and SUPERase•In™ to inhibit RNase A under “preincubation” and “in assay” conditions.** The total yeast RNA assay was performed as described above along with an experimental modification of “preincubation”. For the preincubation assay, the ribonuclease inhibitors were mixed with RNase and incubated for 15 minutes at 22°C. The preincubation mix was then added to the RNA.

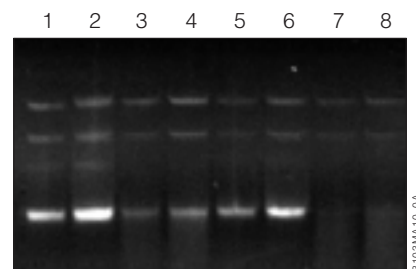
The ability of RNasin® Ribonuclease Inhibitor to effectively inhibit RNase A is not surprising, since the strength of binding of noncompetitive ribonuclease inhibitors to RNase is one of the strongest noncovalent protein interactions known (1). Additional experiments (1) have suggested that the interaction of these RNase inhibitors with RNase is diffusion limited, meaning that the rate of binding is limited only by the diffusion rate of the protein in solution. Therefore, RNasin® Ribonuclease Inhibitor can bind RNase in dilute solution without preincubation of the inhibitor with the enzyme. Since most laboratory techniques require the addition of the ribonuclease inhibitor directly into a reaction tube that already contains RNA and an unknown quantity of RNase, the ability to bind RNase quickly in dilute solutions is essential for an effective ribonuclease inhibitor.

### RNASIN® RIBONUCLEASE INHIBITOR PERFORMS ROBUSTLY IN COMMON LABORATORY TECHNIQUES

**In Vitro Translation:** We tested both SUPERase•In™ and RNasin® Ribonuclease Inhibitor for the ability to protect RNA template from degradation during in vitro translation. Translation of luciferase mRNA was performed in Rabbit Reticulocyte Lysate System<sup>(b,d,e)</sup> (Cat.# L4960) in the presence of inhibitor alone and inhibitor plus RNase A. Adding SUPERase•In™ alone to the translation reaction reduced the translation product made by 27%, indicating that SUPERase•In™ inhibits the translation reaction (Figure 3). Further investigation revealed that the SUPERase•In™ buffer contained the inhibitory reagent. Additionally, SUPERase•In™ conferred only minimal protection of RNA when 10ng of RNase A was added to the translation reaction (Figure 3). RNasin® Ribonuclease Inhibitor did not interfere with the in



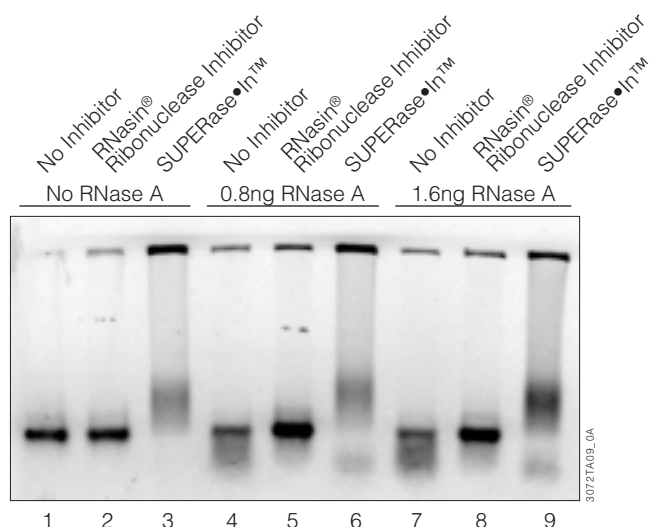
**Figure 3. Comparison of RNasin® Ribonuclease Inhibitor and SUPERase•In™ inhibition of RNase A during in vitro translation.** Luciferase mRNA was translated in Rabbit Reticulocyte Lysate according to the protocol provided in the *Rabbit Reticulocyte Lysate Systems, Nuclease Treated, Technical Manual #TM232*. Luciferase activity was measured using a Turner Luminometer, TD-20e, and the Luciferase Assay System. Eight units of either ribonuclease were used where indicated by (+).



**Figure 4. Comparison of RNasin® Ribonuclease Inhibitor and SUPERase•In™ inhibition of RNase A during in vitro transcription using the Riboprobe® Transcription System according to the protocol provided in the accompanying Technical Manual (#TM016).** RNA products were analyzed on a 1.2% agarose E-Gel™ (Invitrogen) and visualized on a CCD camera made by Alpha Innotech Corporation. Lanes 1 and 2, RNasin® Ribonuclease Inhibitor alone; lanes 3 and 4, RNase A and RNasin® Ribonuclease Inhibitor; lanes 5 and 6, SUPERase•In™ alone; lanes 7 and 8, SUPERase•In™ and RNase A.

vitro translation reaction and also protected the RNA template almost completely from degradation when 10ng of RNase A was added to the reaction (Figure 3).

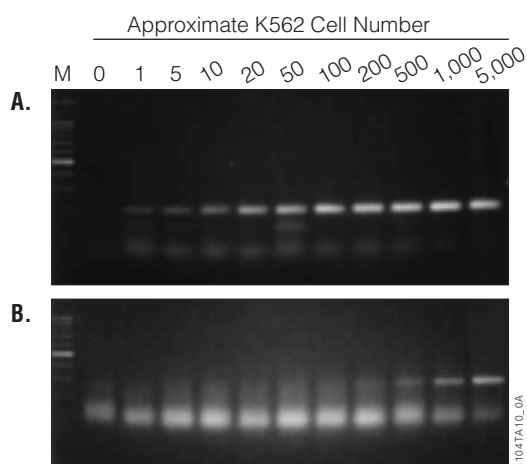
**In Vitro Transcription:** Comparison of SUPERase•In™ and RNasin® Ribonuclease Inhibitor during in vitro RNA synthesis reactions using Riboprobe® Transcription System<sup>(a,b)</sup> (Cat.# P1420) demonstrates that RNasin® Ribonuclease Inhibitor protects RNA from added RNase A. SUPERase•In™ does not provide the same level of protection from RNase A added to the reaction (Figure 4).



**Figure 5. Comparison of RNasin® Ribonuclease Inhibitor and SUPERase•In™ ability to protect RNA from degradation in a nondenaturing gel assay.** A 623bp RNA was labeled with [ $\alpha^{33}$ P]UTP using Promega's RiboMark® Labeling System<sup>(a,b)</sup>. The labeled RNA was incubated in the presence of 5ng RNase A and RNase inhibitors at a concentration of 1 unit/ $\mu$ l for 30 minutes at 37°C. The reactions were analyzed on a 1% agarose TAE gel and visualized using a Molecular Dynamics PhosphorImager®, overnight exposure.

**Gel Fractionation:** Gel fractionation of RNA is performed in a variety of techniques in molecular biology. Such fractionation can be performed in gels that contain a variety of materials such as urea, typically referred to as denaturing gels, or in gels that contain only buffers, typically referred to as nondenaturing gels. In addition, several different RNA sample loading buffers are used by researchers. We have compared the gel fractionation patterns of RNA species in samples treated with RNasin® Ribonuclease Inhibitor or SUPERase•In™ in the presence or absence of RNase A. Although SUPERase•In™ protects RNA from degradation in loading buffers containing urea, it does not appear to provide any protection in the presence of formamide or formaldehyde. RNasin® Ribonuclease Inhibitor, on the other hand, provides protection from RNases in the presence of urea, formamide and formaldehyde. In nondenaturing gel assays comparing RNasin® Ribonuclease Inhibitor and SUPERase•In™ using standard laboratory sample buffers that contain formamide and formaldehyde, RNasin® Ribonuclease Inhibitor protects RNA from degradation. SUPERase•In™ causes an unexplainable gel shift of the RNA in the non denaturing gel assay (Figure 5).

**Single-Cell RT-PCR:** We tested RNasin® Ribonuclease Inhibitor in an application that would be highly sensitive to inhibitors in the product or buffers. We performed experiments to determine if whole cell lysates made from small cell numbers could be successfully used as templates for amplification using Promega's Access RT-PCR System<sup>(a,f)</sup> (Cat.# A1260). We diluted K562 human promyelocytic leukemia cells in PBS such



**Figure 6. Single-Cell RT-PCR using RNasin® Ribonuclease Inhibitor.** **Panel A:** RT-PCR analysis for *bcr-abl* transcript without RNA isolation. K562 human promyelocytic leukemia cells were diluted in sterile 1X PBS such that a theoretical given cell number was present in a 5 $\mu$ l cell volume. The cells were added to 0.5ml tubes containing 5 $\mu$ l of 2X RNasin® freeze medium (2 $\mu$ l Recombinant RNasin® Ribonuclease Inhibitor (40 units/ $\mu$ l) and 18 $\mu$ l buffer (0.15M NaCl, 10mM Tris-HCl [pH 8.0], 5mM DTT). The cells were then quick frozen on dry ice and stored at -70°C. The entire 10 $\mu$ l of lysed cells were added directly to a 50 $\mu$ l RT-PCR using the Access RT-PCR System, following the protocol in the accompanying Technical Bulletin (#TB220). Primers were used at 50pmol each per reaction. The following cycling conditions were used: 48°C for 45 minutes, 95°C for 2 minutes; 40 cycles of 94°C for 30 seconds, 60° for 1 minute, 68°C for 1 minute; final extension at 68°C for 7 minutes, and soak at 4°C. A Perkin Elmer 480 thermocycler was used. Lanes M, 100bp DNA Ladder. **Panel B:** RT-PCR analysis for  $\beta$ -actin transcript without RNA isolation. Reaction conditions and lane representations are as in Panel A.

that a theoretical given cell number was present in a 5 $\mu$ l volume. The cells were then added to 0.5ml microcentrifuge tubes containing 5 $\mu$ l of 2X RNasin® freeze medium (5; see legend to Figure 6) and frozen on dry ice to lyse the cells. The cell lysates were added directly to a 50 $\mu$ l reaction using the Access RT-PCR System (following the protocol in the *Access RT-PCR System Technical Bulletin #TB220*). As seen in Figure 6, the 200bp *bcr-abl* RT-PCR product could be detected by agarose gel electrophoresis from as little as one cell. For  $\beta$ -actin, a 285bp message could be detected from as few as 200 cells (Figure 6). Successful amplification directly from low cell numbers is target specific and some templates may require more than one cell for detectable amplification of a given target.

## CONCLUSIONS

RNA is notoriously susceptible to degradation by nucleases, making RNase inhibitors important reagents for numerous applications. Based on the data described here, there are significant quality differences between RNasin® Ribonuclease Inhibitor and SUPERase•In™. SUPERase•In™ is limited in its ability to bind

RNase quickly in dilute solutions. Additionally, SUPERase•In™ causes unexplainable RNA gel shifts under non-denaturing conditions, although it does show protection of RNA in denaturing gels. SUPERase•In™ buffer also interferes with in vitro translation reactions of luciferase RNA performed in Rabbit Reticulocyte Lysate, and fails to protect RNA from RNase degradation in applications such as in vitro transcription and in vitro translation. RNasin® Ribonuclease Inhibitor is very useful when performing RT-PCR directly with whole cell lysate.

## REFERENCES

1. Shultz, J., Hurst, R. and Betz, N. (2000) *Promega Notes* **77**, 7–13.
2. SUPERase•In™ RNase Inhibitor Certification of Analysis (2000), Ambion Corporation.
3. RNasin® Ribonuclease Inhibitor Product Information Sheet (#9PIN211), Promega Corporation.
4. Lee, F.S. *et al.* (1988) *Biochemistry* **27**, 8545–8553.
5. Klebe, R.J. *et al.* (1996) *BioTechniques* **21**, 1094–1100.

## PROTOCOLS

- ▶ *RNasin® Ribonuclease Inhibitor Product Information* #9PIN211, Promega Corporation. ([www.promega.com/tbs/9pin211/n211abst.html](http://www.promega.com/tbs/9pin211/n211abst.html))
- ▶ *Recombinant RNasin® Ribonuclease Inhibitor Product Information* #9PIN251, Promega Corporation. ([www.promega.com/tbs/9pin251/n251abst.html](http://www.promega.com/tbs/9pin251/n251abst.html))
- ▶ *Rabbit Reticulocyte Lysate System Technical Manual* #TM232, Promega Corporation. ([www.promega.com/tbs/tm232/tm232.html](http://www.promega.com/tbs/tm232/tm232.html))
- ▶ *Luciferase Assay System Technical Bulletin* #TB281, Promega Corporation. ([www.promega.com/tbs/tb281/tb281.html](http://www.promega.com/tbs/tb281/tb281.html))
- ▶ *Riboprobe® In Vitro Transcription Systems Technical Manual* #TM016, Promega Corporation. ([www.promega.com/tbs/tm016/tm016.html](http://www.promega.com/tbs/tm016/tm016.html))
- ▶ *Access RT-PCR System Technical Bulletin* #TB220, Promega Corporation. ([www.promega.com/tbs/tb220/tb220.html](http://www.promega.com/tbs/tb220/tb220.html))

## Ordering Information

Product	Size	Cat.#
RNasin® Ribonuclease Inhibitor <sup>(a)</sup>	2,500 units	N2111
	10,000 units	N2115
Recombinant RNasin® Ribonuclease Inhibitor <sup>(a,b)</sup>	2,500 units	N2511
	10,000 units	N2515

## Related Products

Product	Size	Cat.#
Rabbit Reticulocyte Lysate, Nuclease Treated	5 × 200µl	L4960
Riboprobe® System - T7	System	P1440
Luciferase Assay System	100 assays	E1500
RiboMark® Labeling System	10 reactions	P1550
Access RT-PCR System	100 reactions	A1250

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*E-Gel* is a trademark of Invitrogen. *SUPERase•In* is a trademark of Ambion, Inc. *PhosphorImager* is a registered trademark of Molecular Dynamics.

<sup>(a)</sup>The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

<sup>(b)</sup>U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

<sup>(c)</sup>U.S. Pat. No. 5,552,302, and other patents.

<sup>(d)</sup>U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, 5,814, 471 Australian Pat. No. 649289 and other patents pending.

<sup>(e)</sup>The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.



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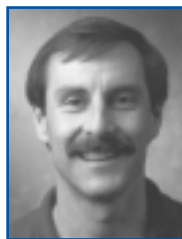
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