RNasin® Ribonuclease Inhibitors: Superior Performance for All of Your RNA Analysis Needs

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Abstract

Over 6,000 papers have cited RNasin® Ribonuclease Inhibitors as the source of RNase protection since Promega introduced RNasin® Inhibitors in 1982, making them the most trusted reagents for RNA protection. RNasin® Inhibitors work by binding strongly to RNases, preventing them from degrading vulnerable RNA molecules during manipulation in the laboratory. RNase inhibitors work in a variety of experimental situations and conditions (1,2). Reliable RNase inhibitors satisfy three major criteria. This article presents these criteria and shows that RNasin® Ribonuclease Inhibitors fulfill them, ensuring that RNases will not interfere with your RNA analysis work.

The purification method and the quality-control assay insure a clean product to protect your RNA.

Criterion 1: RNase Inhibitors Should Protect RNA and Not Introduce RNases

RNase inhibitors must be RNase-free. RNasin® Ribonuclease Inhibitors[a,b,c] form a 1:1 high-affinity complex with RNases, resulting in extremely tight interaction of the inhibitor and the RNase. Therefore, carryover of RNase bound to the RNasin® Inhibitor during purification of the inhibitor is an obvious concern. Figure 1 demonstrates that such carryover can occur in some commercially available preparations of placental RNase inhibitor. Promega has developed extensive purification protocols to separate RNase-bound inhibitor from free inhibitor. Also, Promega has designed thorough quality-control tests to assay specifically for RNase carryover in the purified inhibitor. The QC assay involves heating RNasin® Inhibitor above its normal denaturation temperature, at which point the RNasin® inhibitor:RNase complex is expected to dissociate, and assaying for the reappearance of RNase activity. The purification method and the quality control assay insure a clean product to protect your RNA.

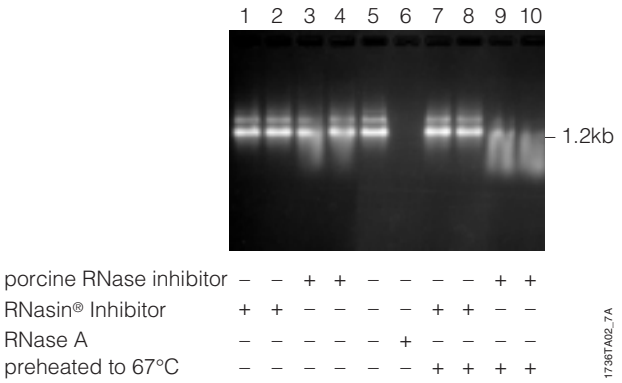

Figure 1. Comparison of RNase and latent RNase activity in Recombinant RNasin® Ribonuclease Inhibitor and porcine RNase inhibitor. Two different amounts (100 units or 200 units) of each inhibitor were incubated with 0.1mg/ml of RNA (1.2kb Kanamycin Positive Control RNA; Cat.# C1381) at 37°C for 60 minutes. As indicated, some samples were preheated to 67°C for 15 minutes (to denature the inhibitor and release any latent RNase) before adding the RNA. The reactants were analyzed on a 1.5% agarose gel with ethidium bromide staining. Lanes 1 and 2, 100 and 200 units, respectively, of Recombinant RNasin® Ribonuclease Inhibitor; lanes 3 and 4, 100 and 200 units, respectively, of porcine RNase inhibitor; lanes 7 and 8, 100 and 200 units, respectively, RNasin® Inhibitor preheated to 67°C; lanes 9 and 10, 100 and 200 units, respectively, of porcine RNase inhibitor preheated to 67°C; lane 5, RNA with no RNase inhibitor added; lane 6, RNA incubated with RNase A in the absence of inhibitor. Full details in reference 3.

Criterion 2: RNase Inhibitors Should Be Compatible with Common Applications

RNase inhibitors must function in the chemical environments common to RNA analysis procedures. Additionally, they should not interfere with the function of commonly performed molecular biology reactions. Promega RNasin® Ribonuclease Inhibitors are extensively tested for compatibility with multiple RNA analysis procedures (Table 1). Compatibility with such enzymes and systems makes RNasin® Inhibitors the ideal inhibitors for all RNA-based applications including microarrays and quantitative, realtime RT-PCR.

Reverse transcription is critical to most methods of RNA analysis. Reverse transcription reactions are often driven to temperatures above the typical 37°C for M-MLV RT or 42°C for AMV RT. M-MLV RT RNase H–, Point Mutant, ImProm-II™ and AMV Reverse Transcriptases can all be used at temperatures of 50°C and above. These temperatures were once thought to fall outside the temperature range of RNasin® Inhibitors. However, recent studies show that there is no reappearance of RNase activity in reactions assembled with RNasin® Plus Ribonuclease Inhibitor and incubated at temperatures as high as 70°C (Figure 2).

Table 1. Promega RNA Analysis Products That Are Compatible with RNasin® Ribonuclease Inhibitors.

Compatible Application		Product	Cat.#
RT-PCR		Access RT-PCR System[a,d]	A1250
		AccessQuick™ RT-PCR System[d]	A1702
First-Strand cDNA Synthesis	✓	Reverse Transcription System[a,b]	A3500
	✓	ImProm-II™ Reverse Transcription System[a,b]	A3800
First- and Second-Strand cDNA Synthesis	✓	Universal RiboClone® cDNA Synthesis System[a,b,e]	C4360
Reverse Transcriptases		M-MLV RT	M1701
		M-MLV RT RNase H–	M5301
		M-MLV RT RNase H– Point Mutant	M3681
		AMV RT	M5101
		ImProm-II™ Reverse Transcriptase	A3802
RNA Polymerases		SP6 RNA Polymerase	P1081
		T3 RNA Polymerase	P2083
		T7 RNA Polymerase	P2075
In vitro Transcription Systems		SP6 Riboprobe® System[a,b]	P1420
		T3 Riboprobe® System[a,b]	P1430
		T7 Riboprobe® System[a,b]	P1440
Large-Scale RNA Production	✓	T7 RiboMAX™ Express RNAi System[a,b,f]	P1700
	✓	T7 RiboMAX™ Express Large Scale RNA Production System[a,b]	P1320
	✓	RiboMAX™ Large Scale RNA Production System—SP6[a,b,g]	P1280
	✓	RiboMAX™ Large Scale RNA Production System—T7[a,b,g,h]	P1300
In vitro Translation		Rabbit Reticulocyte Lysate[a,g,i]	L4960
		Wheat Germ Extract	L4380
Coupled Transcription/Translation		TnT® SP6 Coupled Reticulocyte Lysate System[a,g,i,j]	L4600
		TnT® T3 Coupled Reticulocyte Lysate System[a,g,i,j]	L4950
		TnT® T7 Coupled Reticulocyte Lysate System[a,g,i,j]	L4610
		TnT® SP6 Coupled Wheat Germ Extract System[a,g,i,j]	L4130
		TnT® T3 Coupled Wheat Germ Extract System[a,g,i,j]	L4120
Coupled Transcription/Translation Master Mix	✓	TnT® SP6 Quick Coupled Transcription/Translation System[a,b,g,i,j]	L2080
	✓	TnT® T7 Quick Coupled Transcription/Translation System[a,b,g,i,j]	L1170
	✓	TnT® T7 Quick for PCR DNA[a,b,j]	L5540

✓Denotes that system includes Recombinant RNasin® Ribonuclease Inhibitor either as a separate tube or within the Master Mix.

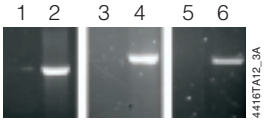

Figure 2. Protection from RNase at 70°C. Separate tubes of RNasin® Plus and RNase (lanes 1, 3, and 5) were heated to 70°C for 15 minutes. RNasin® Plus and RNase were combined and then heated to 70°C for 15 minutes (lanes 2, 4 and 6). To each set of reactions, varied amounts of Luciferase Control RNA[a,g] (Cat.# L4561) were added: 1µg (lanes 1 and 2); 100ng (lanes 3 and 4); or 10 ng (lanes 5 and 6). The reactions were either held at 37°C (lanes 3–6) for 1 hour or directly used in an RT-PCR (lanes 1 and 2) to amplify the entire 1.8kb transcript with the AccessQuick™ RT-PCR System[d] (Cat.# A1702). The gel shows the amplified product from the RT-PCR. Lanes 1 and 2 used 40u of RNasin® Plus RNase Inhibitor and 20ng RNase A (Cat.# A7973). Lanes 3–6 used 400u of RNasin® Plus and 1.25µg of a rat liver protein extract (Sigma Cat.# L-1380) dissolved in water to 0.5µg/µl (reference 2). Additional information about the stability of RNasin® Plus RNase Inhibitor is available from reference 2.

Criterion 3: RNase Inhibitors Must Work Rapidly

All companies and researchers strive to create RNase-free environments, designing RNA purification methods that eliminate RNases. However, RNases may be introduced into a reaction from an unexpected source. Therefore, RNase inhibitors must work instantly with no appreciable lag between introduction of an RNase and inhibition of that RNase. RNasin® Inhibitors have an extremely high affinity for RNases ($K_i = 4 \times 10^{-14}$M) and thus can inhibit almost instantaneously. Inhibitors with lower affinities for RNase, such as SUPERase•In™, cannot act as quickly, especially against trace amounts of RNase. Figure 3 presents data that show a lag between the time the RNase is introduced and the time that RNase is inhibited with the lower affinity RNase inhibitors.

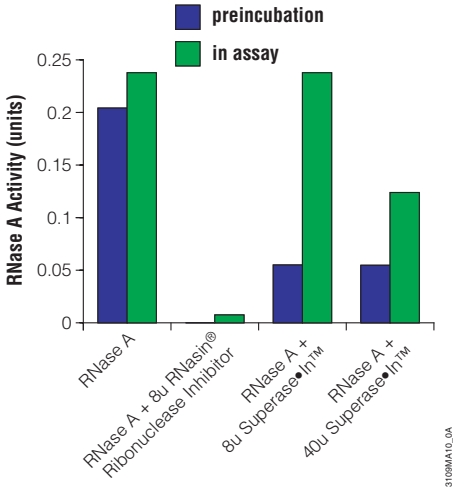

Figure 3. Comparison of RNasin® Ribonuclease Inhibitor and SUPERase•In™ inhibitor to inhibit RNase A under "preincubation" and "in assay" conditions. Total yeast RNA was incubated in the presence of 5ng RNase A for 15 minutes at 37°C in 0.5ml of reaction mix containing 50mM MOPS and 5mM $MgCl_2$ (pH 6.5). RNase inhibitors were present or absent as indicated in the graph. After incubation 0.5ml 10% TCA was added to stop the reaction and to precipitate the large RNA molecules. An OD_{280} measurement was taken of the TCA-soluble material. 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 (4).

RNasin® Ribonuclease Inhibitors are ideal for any RNA-based application because they meet all of the criteria discussed here. They protect RNA and are themselves free from RNase contamination. They are compatible with a wide variety of RNA-based applications, and if an RNase is introduced into your experiment from an outside source, the RNase is rapidly neutralized. RNasin® Inhibitors insure that the presence of an RNase will not prevent you from getting the results you need.

References

1. Shultz, J., Hurst, R. and Betz, N. (2001) RNasin® Ribonuclease Inhibitor Part I: Characterization of the protein. *Promega Notes* **77**, 8–11.
2. Andrew, C., Huang, F. and Shultz, J. (2003) RNasin® Plus RNase Inhibitor: New protein for high temperature RNase Inhibition. *eNotes* (http://www.promega.com/enotes/applications/ap0049_tabs.htm)
3. Schink, M., Mei, B. and Lepinske, M. (1997) A comparison of ribonuclease inhibitors. *Promega Notes* **61**, 30–2.
4. Shultz, J., *et al.* (2001) RNasin® Ribonuclease Inhibitor Part II: A tale of two proteins. *Promega Notes* **77**, 12–5.

Protocols

- *Recombinant RNasin® Ribonuclease Inhibitor Product Information Sheet* 9PIN251, Promega Corporation. (www.promega.com/tbs/9pin251/9pin251.html)
- *RNasin® Plus RNase Inhibitor Product Information Sheet* 9PIN261, Promega Corporation. (www.promega.com/tbs/9pin261/9pin261.html)

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Product Manager

Ordering Information

Product	Size	Cat.#
Recombinant RNasin® Ribonuclease Inhibitor[a,b]	2,500u	N2511
	10,000u	N2515
RNasin® Plus RNase Inhibitor[c]	2,500u	N2611
	10,000u	N2615

For Laboratory Use.

[a]U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

[b]U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

[c]Patent Pending.

[d]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.

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[f]This product is covered under license from Carnegie Institution of Washington. Commercial use of this product may require a separate license from Carnegie.

[g]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. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

[h]The RiboMAX™ Large Scale RNA Production System—T7 (Cat.# P1300) is covered by U.S. Pat. Nos. 5,256,555, 6,586,218, 6,586,219 and other pending patents and is sold under a license from Ambion, Inc. It is intended for research use only. Parties wishing to use this product for other applications should contact Ambion, Inc.

[i]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents and patents pending.

[j]U.S. Pat. Nos. 5,324,637 and 5,492,817, Australian Pat. No. 660329 and other patents.