Certificate of Analysis

RNasin® Plus RNase Inhibitor:

Part No.	Size (units)
N2611	2,500
N2615	10,000

Part# 9PIN261
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Description: RNasin® Plus RNase Inhibitor[a] is a recombinant mammalian RNase inhibitor purified by a combination of ion exchange and hydrophobic interaction chromatography to yield a physical purity >90%. The protein is capable of inhibiting eukaryotic RNases (e.g., RNase A and RNase B) similar to human placental RNase inhibitor. Certain cysteine residues present in human placental RNase inhibitor have been implicated in the oxidation sensitivity of that enzyme (1). RNasin® Plus RNase Inhibitor does not contain these cysteine residues, which makes it more resistant to oxidative damage. RNasin® Plus RNase Inhibitor forms a stable complex with RNases, inactivating the RNase. This complex is stable at temperatures up to 70°C for at least 15 minutes. RNasin® Plus RNase Inhibitor has been tested in RT-PCR and is compatible with enzymes such as AMV, M-MLV and ImProm-II™ Reverse Transcriptases or *Taq* and *Tfl* DNA polymerases. RNasin® Plus RNase Inhibitor also has been tested and found to be compatible with quantitative, real-time RT-PCR reactions in a TaqMan® Assay.

Enzyme Storage Buffer: RNasin® Plus RNase Inhibitor is supplied in 20mM HEPES-KOH (pH 7.6), 50mM KCl, 8mM DTT, 50% (v/v) glycerol.

Source: *E. coli* cells expressing a recombinant clone.

Storage Conditions: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. The expiration date is on the Product Information Label.

Unit Definition: One unit is defined as the amount of RNasin® Plus RNase Inhibitor required to inhibit the activity of 5ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2′,3′-cyclic monophosphate by RNase A. The unit concentration is on the Product Information Label.

Usage Notes: RNasin® Plus RNase Inhibitor is active over a broad pH range. Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use. If using RNasin® Plus RNase Inhibitor prior to using denaturants (e.g., temperatures >50°C, urea, SDS, etc.), add additional RNasin® Plus RNase Inhibitor following removal of denaturants to provide protection against exogenous RNases that may be present in other reagents added to your RNA sample.

Table 1. Properties of RNasin® Plus RNase Inhibitor.

Property	Comment
Activity	Inactivates RNase by noncovalent binding
Type of Inhibition	Noncompetitive
Binding Ratio with RNase A	1:1
Calculated Molecular Weight	49,905 daltons
Calculated Isoelectric Point	4.6
Amount to Use	1 unit of inhibitor per microliter of solution

Table 2. Targets of RNasin® Plus RNase Inhibitor.

Inhibits	Compatible With
RNase A	*Taq* DNA polymerase
RNase B	*Tfl* DNA polymerase
RNases present in rat liver extract	AMV and M-MLV reverse transcriptases
	ImProm-II™ Reverse Transcriptase

Quality Control Assays

Contaminant Activity

RNase Assays: To test for the presence of *E. coli* RNase activity, 1µg of RNA is incubated with 200 units of RNasin® Plus RNase Inhibitor for 1 hour at 37°C. The RNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of degradation. To test for the presence of latent *E. coli* RNase activity, RNasin® Plus RNase Inhibitor is heated at 67°C for 15 minutes, and the equivalent of 200 units is incubated with 1µg of RNA for 1 hour at 37°C. The RNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of RNA degradation.

DNase Assay: To test for DNase activity, 50ng of radiolabeled DNA is incubated with 200 units of RNasin® Plus RNase Inhibitor for 1 hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is <3% release.

Endonuclease Assay: To test for endonuclease activity, 1µg of supercoiled plasmid DNA is incubated with 200 units of RNasin® Plus RNase Inhibitor for 2 hours at 37°C in Promega Restriction Enzyme Buffer B (6mM Tris-HCl [pH 7.5], 50mM NaCl, 6mM $MgCl_2$, 1mM DTT). Following incubation, the supercoiled (Type I) DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Physical Purity: The purity is ≥90% as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

Reference

1. Kim, B.M., Schultz, L.W. and Raines, R.T. (1999) Variants of ribonuclease inhibitor that resist oxidation. *Protein Sci.* **8**, 430–4.

Signed by:

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Usage Information

1. Target RNA and Primer Denaturation

The following protocol describes RNA template denaturation prior to assembly of a 20–50µl RT-PCR. Denaturation of target RNA prior to RT-PCR increases cDNA yields by decreasing RNA secondary structure. RNasin® Plus RNase Inhibitor will bind to exogenous RNases that could be introduced in this step and remain bound to them throughout the target denaturation process.

1. Place sterile, thin-walled reaction tubes on ice. Thaw the experimental RNA on ice, and return any unused portion to the freezer as soon as aliquots are taken.
2. Combine the following on ice:

RNasin® Plus RNase Inhibitor		1µl
experimental RNA (typically up to 1µg)		Xµl
cDNA synthesis primer		Yµl
oligo(dT)	0.5µg/reaction	
or random hexameric primer	0.5µg/reaction	
or gene-specific primers	10–20pmol/reaction	
nuclease-free water		Zµl
Final volume		5–10µl

3. Close each tube of RNA/primer tightly. Place tubes into a preheated 70°C heat block for 5–10 minutes. Immediately chill in ice-water for at least 5 minutes. Centrifuge each tube for 10 seconds in a microcentrifuge to collect the liquid. Keep the tubes closed and on ice until the reverse transcription reaction mix has been added.
4. Proceed to either coupled or uncoupled RT-PCR as directed by your system.

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