RNasin® Plus RNase Inhibitor:

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Size (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2611</td>
<td>2,500</td>
</tr>
<tr>
<td>N2615</td>
<td>10,000</td>
</tr>
</tbody>
</table>

**Description:** RNasin® Plus RNase Inhibitor 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 Transcripts or Taq and T7 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.

**Properties of RNasin**

- **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.**

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

**Table 2. Targets of RNasin® Plus RNase Inhibitor.**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Compatible With</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase A</td>
<td>Taq DNA polymerase</td>
</tr>
<tr>
<td>RNase B</td>
<td>T7 DNA polymerase</td>
</tr>
<tr>
<td>RNases present in rat liver extract</td>
<td>AMV and M-MLV reverse transcriptases</td>
</tr>
<tr>
<td></td>
<td>ImProm-II™ Reverse Transcriptase</td>
</tr>
</tbody>
</table>

**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 MgCl2, 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**


Signed by:

R. Wheeler, Quality Assurance

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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.