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

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

Source: E. coli cells expressing a recombinant clone.

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

Usage Notes: Recombinant RNasin® Ribonuclease 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.

Table 1. Properties of Recombinant RNasin™ Ribonuclease Inhibitor.

<table>
<thead>
<tr>
<th>Property</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>Inactivates RNase by noncovalent binding</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>49,847 daltons</td>
</tr>
<tr>
<td>Type of inhibition</td>
<td>Noncompetitive (3)</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>pI 4.7</td>
</tr>
<tr>
<td>pH activity range</td>
<td>pH 5.5–9 (4)</td>
</tr>
<tr>
<td>Binding ratio with RNase A</td>
<td>1:1 (3)</td>
</tr>
<tr>
<td>Constant for binding inhibition</td>
<td>$K_i = 4 \times 10^{-14} \text{M}$ (3,4)</td>
</tr>
<tr>
<td>Amount to use</td>
<td>1 unit of inhibitor per microliter of solution</td>
</tr>
<tr>
<td>Reaction conditions to avoid</td>
<td>Temperatures &gt;50°C, uren, SDS, other denaturants</td>
</tr>
</tbody>
</table>

Table 2. Effectiveness of Recombinant RNasin® Ribonuclease Inhibitor Against Selected Nucleases.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Does Not Inhibit</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase A</td>
<td>RNase T1</td>
</tr>
<tr>
<td>RNase B</td>
<td>S1 Nuclease</td>
</tr>
<tr>
<td>RNase C</td>
<td>RNase from Aspergillus sp.</td>
</tr>
<tr>
<td>Human placental RNase</td>
<td>RNase H, RNase ONE™, Ribonuclease, TagDNA polymerase, ImProm-II™, AMV or M-MLV Reverse Transcriptase, SP6, T7 or T3 RNA polymerase</td>
</tr>
</tbody>
</table>

Quality Control Assays

RNSase Assays: To test for the presence of RNase activity, 1µg of RNA is incubated with 200 units of Recombinant RNasin® Ribonuclease Inhibitor for 1 hour at 37°C, and the RNA is then visualized on an ethidium bromide-stained agarose gel to verify the absence of degradation. To test for the presence of latent RNase activity, RNasin® Ribonuclease Inhibitor is heat-denatured at 67°C for 15 minutes and the equivalent of 200 units are then incubated with 1µg of RNA for 1 hour at 37°C. The RNA is then visualized on an ethidium bromide-stained agarose gel. No RNA degradation is detected.

DNase Assay: To test for DNase activity, 50ng of radiolabeled DNA is incubated with 200 units of Recombinant RNasin® Ribonuclease 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 Recombinant RNasin® Ribonuclease 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.

Signed by: R. Wheeler, Quality Assurance
1. Description

RNasin® Ribonuclease Inhibitors have broad-spectrum RNase inhibitory properties, including inhibition of eukaryotic RNases of the neutral type (1; see Table 1). The 50kDa protein exerts its inhibitory effect by noncovalently binding to RNases at a 1:1 ratio. The Kᵢ value for the binding of RNasin® Ribonuclease Inhibitor to RNase (e.g., RNase A) is approximately 10⁻¹⁴M (2–4). Typically, antibodies by comparison have a binding constant of 10⁻⁶–10⁻¹⁰M. In addition, the kinetics of association for RNasin® Ribonuclease Inhibitor is very rapid, ensuring immediate complexing and inhibition of RNase. Promega offers two different preparations: Natural RNasin® Ribonuclease Inhibitor and Recombinant RNasin® Ribonuclease Inhibitor. These products are purified by using a combination of ion exchange and affinity chromatography. They are devoid of DNA exonuclease and endonuclease activity and RNase activity. In addition to its ability to inhibit RNase activity, RNasin® Ribonuclease Inhibitor has been shown to inhibit angiogenesis induced by angiogenin (S).

Recombinant RNasin® Ribonuclease Inhibitor offers the researcher an extra level of assurance of purity and consistency. Isolated from a recombinant E. coli strain, the N-terminus is an unblocked serine residue.

General Considerations: Since ribonucleases typically retain activity under denaturing conditions, care must be taken to avoid denaturing RNasin® Ribonuclease Inhibitor molecules that have complexed with ribonuclease. To prevent the release of active ribonuclease, temperatures greater than 50°C and high concentrations of urea or other denaturing agents should be avoided. RNasin® Ribonuclease Inhibitors are active over a broad pH range. If diluted and stored for extended periods of time, include DTT (minimum concentration 1mM).

2. Standard Applications

Both Recombinant and Natural RNasin® Ribonuclease Inhibitor can be used interchangeably in in vitro transcription and translation applications, described below. For more information on systems and protocols for in vitro transcription, please request the Riboprobe® In Vitro Transcription Systems Technical Manual #TM016.

A. Transcription In Vitro (unlabeled RNA)

The standard in vitro transcription assay below uses RNasin® Ribonuclease Inhibitor at a final concentration of 1u/µl. With appropriate modifications, this reaction can be used for in vitro transcription analysis in a variety of experimental applications.

- **5X transcription buffer**
- **DTT, 100mM**
- **RNasin® Ribonuclease Inhibitor**
- **ATP, GTP, CTP and UTP, 2.5mM each**
- **linearized plasmid DNA, 2–5µg in water or TE buffer**
- **RNA polymerase; SP6, T3 or T7**
- **Incubate for 60–120 minutes at 37–40°C.**
- **Final volume of 50µl**
- **Nuclease-free water to a final volume of 100µl**

Incubate for 60–120 minutes at 37–40°C.

B. Transcription In Vitro (³²P-labeled RNA probes)

- **5X transcription buffer**
- **DTT, 100mM**
- **RNasin® Ribonuclease Inhibitor**
- **ATP, GTP and UTP, 2.5mM each**
- **CTP, 100µM**
- **Linearized template DNA, 0.2–1.0µg/ml in water or TE buffer**
- **RNA polymerase; SP6, T3 or T7**
- **Incubate for 60 minutes at 37–40°C.**

Incubate for 60 minutes at 37–40°C.

**C. Translation In Vitro**

Include RNasin® Ribonuclease Inhibitor in standard and coupled in vitro translation systems to ensure protection of RNA substrates.

Sample Reaction using Rabbit Reticulocyte Lysate for In Vitro Translation:

- **Rabbit Reticulocyte Lysate**
- **Nuclease-free water**
- **RNasin® Ribonuclease Inhibitor**
- **Amino Acid Mixture Minus Methionine, 1mM**
- **[³²S]Methionine (1,200Ci/mmol) at 10µCi/ml**
- **DNA template**
- **Final volume of 50µl**

Incubate for 60 minutes at 30°C.

Sample Reaction using the TNT® Reticulocyte Lysate or Wheat Germ Extract Systems for Coupled Transcription/Translation:

- **TNT® Rabbit Reticulocyte Lysate or Wheat Germ Extract**
- **Nuclease-free water**
- **RNasin® Ribonuclease Inhibitor**
- **DNA template**
- **Final volume of 50µl**

Incubate for 60–120 minutes at 30°C.

3. Composition of Buffers and Solutions

- **5X transcription buffer**
  - 200mM Tris-HCl (pH 7.5)
  - 30mM MgCl₂
  - 10mM spermidine
  - 50mM NaCl
- **1X TE buffer**
  - 10mM Tris-HCl (pH 8.0)
  - 1mM EDTA

4. References