**Product Safety Note:** Promega qualified and verified the RNasin® Ribonuclease Inhibitor manufacturing process for its viral clearance ability when challenged with significant titers of live viruses. Promega opted for this approach in place of tests on individual lots of finished product with probes for specific viruses.

No known test methods can offer a guarantee that products derived from human placenta will not transmit infectious agents. Instead of lot-by-lot testing with a handful of specific analytes, Promega established process controls to provide our customers a high degree of assurance that the final product will most likely be free of infectious viruses. The proprietary purification procedure for RNasin® Ribonuclease Inhibitor was qualified by an independent biosafety testing laboratory for its capability to eliminate contaminating viruses at each of the multiple purification steps. The testing laboratory used an industry standard, viral-challenge protocol with a representative panel of human and animal viruses (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, or ICH, 1997. Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin).

The validation study showed that when the purification process is challenged with high titers of live virus, including human HIV-1 and HAV viruses, the process clears viral loads by many thousandfold. Under the normal conditions we use for purifying placental material, no viral titers are detectable.

Customers are nonetheless advised to use universal safety precautions in the handling and use of this product as if it were infectious.

**RNasin® Ribonuclease Inhibitor:**

### Quality Control Assays

#### Contaminant Activity

**RNase Assays:** To test for the presence of RNase activity, 1µg of RNA is incubated with 200 units of native 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 is incubated with 1µg of RNA for 1 hour at 37°C. The RNA is then visualized on an ethidium bromide-stained agarose gel to verify the absence of degradation.

**DNase Assay:** To test for DNase activity, 50ng of radiolabeled DNA is incubated with 200 units of 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 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.

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**Signed by:**

R. Wheeler, Quality Assurance
1. Description

Natural and Recombinant 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). Promega offers two different preparations: Natural RNasin® Ribonuclease Inhibitor and Recombinant RNasin® Ribonuclease Inhibitor. These products are purified using a combination of ion exchange and affinity chromatography, and Natural and Recombinant RNasin® Ribonuclease Inhibitors 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, basic fibroblast growth factor (bFGF) and sodium orthovanadate (5,6). Moreover, RNasin® Ribonuclease Inhibitor reduced neovascularization evoked by implanted tumor cells and significantly inhibited tumor growth in a murine model (6).

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.

<table>
<thead>
<tr>
<th>Table 1. Properties of RNasin® Ribonuclease Inhibitor.</th>
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<tbody>
<tr>
<td>Activity</td>
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<tr>
<td>Molecular weight</td>
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<td>Type of inhibitor</td>
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<td>Isoelectric point</td>
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<td>pH activity range</td>
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<td>Binding ratio with RNase A</td>
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<td>Constant for binding inhibition</td>
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<tr>
<td>Amount to use</td>
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<td>Reaction conditions to avoid</td>
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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 1 unit/ml. 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, 2–5ug in H₂O or TE buffer
- RNA polymerase, SP6, T3 or T7
- 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, 0.2–1.0mg/ml in H₂O or TE buffer
- [³²P]CTP, 50Ci at 10mCi/ml
- RNA polymerase, SP6, T3 or T7
- nuclease-free water to a final volume of 20µl

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 35µl
- nuclease-free water 7µl
- RNasin® Ribonuclease Inhibitor 40µl
- Amino Acid Mixture Minus Methionine, 1mM 1µl
- [³⁵S]methionine, (1,200Ci/mmol) at 10mCi/ml 4µl
- RNA template in H₂O 2µg
- 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 25µl
- RNA template in H₂O 2µg
- RNasin® Ribonuclease Inhibitor 40µl/µl 40µl
- DNA template 1µg
- Nuclease-free water to a 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-Cl (pH 8.0)
- 1mM EDTA

4. References