Certificate of Analysis

RNasin® Ribonuclease Inhibitor:

Part No.	Size (units)
N211A	2,500
N211B	10,000

Part# 9PIN211

Revised 4/18

Description: Natural RNasin® Ribonuclease Inhibitor is isolated from human placenta. Preparations may contain multiple polymorphic inhibitors that differ slightly in amino acid sequence. The N-terminus is blocked. The manufacturing protocol used results in a product that tests negative for the presence of HIV-1 and Hepatitis A, B and C. **Caution: No known test methods can offer assurance that products derived from human placental tissue will not transmit infectious agents. All such products should be treated as infectious.**

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.

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

Source: Human placenta.

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.

Unit Definition: One unit is defined as the amount of 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: RNasin® Ribonuclease Inhibitor is active over a broad pH range but *requires a minimum of 1mM dithiothreitol to maintain activity.* Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

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 $MgCl_2$, 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.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

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 K_i value for the binding of RNasin® Ribonuclease Inhibitor to RNase (e.g., RNase A) is approximately 10^{-14}M (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 1. Properties of RNasin® Ribonuclease Inhibitor.

Property	Comment
Activity	Inactivates RNase by noncovalent binding
Molecular weight	~49kDa
Type of inhibition	Noncompetitive (3)
Isoelectric point	pI 4.7
pH activity range	pH 5–9 (highest activity at pH 7–8) (4)
Binding ratio with RNase A	1:1 (3)
Constant for binding inhibition	$K_i = 4 \times 10^{-14}$M (3,4)
Amount to use	1 unit of inhibitor per microliter of solution
Reaction conditions to avoid	Temperatures >50°C, urea, SDS, other denaturants

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

Inhibits	Does Not Inhibit
RNase A	RNase T1
RNase B	S1 Nuclease
RNase C	RNase from *Aspergillus sp.*
human placental RNase	RNase H, RNase ONE™ Ribonuclease, *Taq* DNA polymerase, AMV reverse transcriptase, M-MLV reverse transcriptase, SP6, T3 or T7 RNA polymerase

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	20µl
DTT, 100mM	10µl
RNasin® Ribonuclease Inhibitor	100u
ATP, GTP, CTP and UTP, 2.5mM each*	20µl
linearized plasmid DNA, 2–5µg in H_2O or TE buffer	2µl
RNA polymerase; SP6, T3 or T7	0–50u
nuclease-free water to a final volume of	100µl

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

*Prepare by mixing equal volumes of four 10mM rNTP stocks.

B. Transcription in vitro (^{32}P-labeled RNA probes)

5X transcription buffer	4µl
DTT, 100mM	2µl
RNasin® Ribonuclease Inhibitor	20u
ATP, GTP and UTP, 2.5mM each**	4µl
CTP, 100µM	2.4µl
linearized template DNA, 0.2–1.0mg/ml in H_2O or TE buffer	1µl
[α-^{32}P]CTP, 50µCi at 10mCi/ml	5µl
RNA polymerase, SP6, T3 or T7	1µl
nuclease-free water to a final volume of	20µl

Incubate for 60 minutes at 37–40°C.

**Mix 1 volume of H_2O with 1 volume each of 10mM ATP, GTP and UTP stock solutions.

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	40u
Amino Acid Mixture Minus Methionine, 1mM	1µl
[^{35}S]methionine, (1,200Ci/mmol) at 10mCi/ml	4µl
RNA template in H_2O	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
TnT® Reaction Buffer	2µl
TnT® T3, T7 or SP6 RNA Polymerase	1µl
Amino Acid Mixture Minus Methionine, 1mM	1µl
[^{35}S]methionine (1,000Ci/mmol) at 10mCi/ml	4µl
RNasin® Ribonuclease Inhibitor, 40u/µl	40u
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	$MgCl_2$
10mM	spermidine
50mM	NaCl

1X TE buffer

10mM	Tris-HCl (pH 8.0)
1mM	EDTA

4. References

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