

CERTIFICATE OF ANALYSIS

Product Name: RNHI RNase Inhibitor

Cat. No.: PBIRH0030

Lot. No.:

Manufacturing Date:

Expiration Date:

Unit definition: One unit is defined as the amount of RNHI RNase Inhibitor required to inhibit the activity of 5 ng of RNaseA by 50%.

Optimal Storage Temp: - 20°C ± 5°C.

Concentration: 20 U/μL

Quantity 2,000 Units

Source: Recombinant *E. coli* strain.

Storage buffer: 20 mM HEPES-KOH, 50 mM KCl, 0.1 mM EDTA,
50% glycerol, 8 mM DTT, 0.05% Tween20, pH7.6

Parameter	Specifications	Results
Appearance	Clear, colorless	Corresponds
<i>E.coli</i> genomic DNA contamination	≤ 0.1 pg/100U	Corresponds
Ribonucleases Activity	Not detectable	Corresponds
Latent Ribonucleases Activity	Not detectable	Corresponds
Non-Specific DNase Activity	Not detectable	Corresponds
Protein Purity	≥ 90%	Corresponds

Signature of Quality Assurance Supervisor, Date

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QUALITY CONTROL ASSAY

- ✓ ***E.coli* genomic DNA Contamination:** 100 units RNHI RNase Inhibitor denatured and assessed using QuantStudio Absolute Q Digital PCR System, (ThermoFisher), for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.
- ✓ **Ribonucleases Activity:** To test the presence of *E.coli* RNase activity, 1µg of MS2 RNA(Bacteriophage) is incubated with 160 units of RNHI RNase Inhibitor for 1 hour at 37°C, and the RNA is visualized on a SYBR™ Gold-stained agarose gel to verify the absence of degradation.
- ✓ **Latent Ribonucleases Activity:** To test the presence of latent *E.coli* RNase activity, RNHI RNase Inhibitor is inactivated at 70°C for 15 minutes, and the equivalent of 160 units is incubated with 1µg of MS2 RNA(Bacteriophage) for 1 hour at 37°C. The RNA is visualized on a SYBR™ Gold-stained agarose gel to verify the absence of RNA degradation.
- ✓ **Non-Specific DNase Activity:** 1ug of Lambda-HindIII is incubated with 160 units of RNHI RNase Inhibitor for 1hour at 37°C, and the DNA pattern is then visualized on an ethidium bromide-stained agarose gel to verify the absence of DNA fragments degradation.
- ✓ **Protein Purity Assay:** RNHI RNase Inhibitor is $\geq 90\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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Product Information

1. Description

RNH1 Ribonuclease Inhibitor is a recombinant protein of murine origin which specifically inhibits RNases A, B and C but not RNase H, RNase 1, RNase T1, and S1 nuclease. In addition, no inhibition of polymerase activity is observed and is compatible with RT-PCR enzymes such as M-MLV Reverse Transcriptase and Taq DNA polymerase

2. Application

RNHI RNase Inhibitor is used to:

- Protect mRNA in cDNA synthesis reactions, RT-PCR, *in vitro* transcription/translation system, RNase Protection Assay, *in vitro* RNA synthesis.
- Inhibit RNases during RNA isolation and purification.
- Help prepare RNase-free antibodies.

3. Recommended Use

One microliter (20 units) RNHI RNase inhibitor in total 20 µl of reaction mixture is suggested to protect mRNA and improve total cDNA yield in the first strand cDNA synthesis. RNHI RNase inhibitor is active at pH of 6.0-9.0. Should **NOT** be greater than 50°C to prevent inactivation of RNHI RNase inhibitor.