**Protector RNase Inhibitor**

**Cat. No. 03 335 399 001**  
2,000 U  
**Cat. No. 03 335 402 001**  
10,000 U (5 × 2,000 U)

Special Quality for Molecular Biology

1. **What this Product Does**

**Contents**

<table>
<thead>
<tr>
<th>Vial</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protector RNase Inhibitor (40 U/µl)</td>
<td>• 50 µl (2,000 U pack size)</td>
</tr>
<tr>
<td></td>
<td>• 5 vials of 50 µl (10,000 U pack size)</td>
</tr>
<tr>
<td>Storage buffer: 20 mM Hepes-KOH, 50 mM KCl, 8 mM dithiothreitol, 50% glycerol (v/v), pH approximately 7.6 (+4°C)</td>
<td></td>
</tr>
</tbody>
</table>

**Storage and Stability**

Stable at —15 to —25°C until the control date printed on the label.

**Applications**

Protector RNase Inhibitor inactivates a wide spectrum of RNases, including:

- RNase A
- RNase B
- RNase T2

Thus, Protector RNase Inhibitor can help prevent RNase degradation in any application where RNases could cause problems. For instance, it can:

- protect mRNA during cDNA synthesis reactions, RT-PCR (in conventional thermal cyclers and qPCR systems), or *in vitro* transcription/translation reactions
- protect viral RNA during *in vitro* virus replication
- inhibit RNases during RNA isolation and purification
- be used in RNase protection assays
- help prepare RNase-free antibodies

Protector RNase Inhibitor does not interfere with enzymes commonly used to prepare or analyze RNA, for example:

**Application**  
RT-PCR  
cDNA synthesis  
real-time qPCR  
in *in vitro* transcription/translation

**Products**

- Transcriptor Reverse Transcriptase, when used with Taq DNA Polymerase, FastStart Taq DNA Polymerase, Expand High Fidelity PCR System, Titan One Tube RT-PCR System
- cDNA Synthesis System
- LightCycler® Reagents and Kits
- T7 RNA Polyomerase (in wheat germ lysate)

* available from Roche Applied Science

2. **How To Use this Product**

**Working concentration**

Use the following table to determine the optimal Protector RNase Inhibitor concentration.

<table>
<thead>
<tr>
<th>Application</th>
<th>One-step RT-PCR</th>
<th>Two-step RT-PCR</th>
<th><em>in vitro</em> transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 to 10 U</td>
<td>25 to 50 U</td>
<td>20 U</td>
</tr>
</tbody>
</table>

⚠️ You may use higher concentrations of Protector RNase Inhibitor in RT-PCR if you suspect that RNase contamination causes certain samples to be difficult to amplify. The inhibitor does not interfere with the reaction. In a test system, even a 16-fold higher concentration of inhibitor did not interfere with RT-PCR.

3. **Additional Information on this Product**

**Source**

Rat lung; recombinant product is produced in *E. coli*.

**Storage buffer**

20 mM Hepes-KOH, 50 mM KCl, 8 mM dithiothreitol, 50% glycerol (v/v), pH approximately 7.6 (+4°C)

**Inactivation**

Severe denaturing conditions (such as temperatures above +65°C) inactivate the inhibitor.

**Product characteristics**

<table>
<thead>
<tr>
<th>Volume activity</th>
<th>40 U/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>approximately 50 kDa</td>
</tr>
<tr>
<td>Purity (SDS-PAGE)</td>
<td>&gt; 95%; only one visible product band</td>
</tr>
<tr>
<td>Bioburden</td>
<td>&lt; 50 cfu/ml</td>
</tr>
<tr>
<td>DNA content</td>
<td>&lt; 100 pg/mg</td>
</tr>
<tr>
<td>Active pH range</td>
<td>pH 5.0 to pH 9.0</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>pH 4.5</td>
</tr>
<tr>
<td>Active temperature range</td>
<td>+25°C to +55°C (enzyme retains partial activity at +60°C)</td>
</tr>
</tbody>
</table>

**Unit assay**

One unit of Protector RNase Inhibitor is defined as the amount of protein required to inhibit 50% of the activity of 1 µg RNase A. Activity is measured according to Blackburn (1) as ability to inhibit hydrolysis of cyclic cytidine-2′ : 3′-monophosphoric acid. Under assay conditions, 200 U of Protector RNase Inhibitor inhibits 50% of the activity of 1 µg RNase A.
Quality Control

Each lot of Protector RNase Inhibitor is function tested with the Titan One Tube RT-PCR Kit* on a LightCycler® Carousel-based System*. Protector RNase Inhibitor is also tested for contaminating activities as described below.

Test buffer: 50 mM Tris-HCl, 10 mM MgCl₂, 0.1 mM EDTA, 7 mM β-mercaptoethanol; pH 7.5 (+37°C).

Absence of endonucleases: 1 µg DNA Molecular Weight Marker II is incubated with Protector RNase Inhibitor for 1 hour at +37°C in a final volume of 50 µl. Incubation with up to 400 U Protector RNase Inhibitor does not show degradation of DNA Molecular Weight Marker II.

Absence of nicking activity: 1 µg supercoiled pBR322 DNA is incubated with Protector RNase Inhibitor for 1 hour at +37°C in a final volume of 50 µl. Incubation with up to 400 U Protector RNase Inhibitor does not show relaxation of supercoiled DNA.

Absence of ribonuclease (1): 5 µg of MS2 RNA is incubated with Protector RNase Inhibitor for 1 hour at +37°C in a final volume of 50 µl. Incubation with up to 400 U Protector RNase Inhibitor does not show degradation of MS2 RNA.

Absence of ribonuclease (2): 5 µg of MS2 RNA is incubated with Protector RNase Inhibitor for 1 hour at +37°C, then 10 min at +65°C in a final volume of 50 µl. Incubation with up to 160 U Protector RNase Inhibitor does not show degradation of MS2 RNA.

4. Supplementary Information

4.1 Conventions

Symbols

In this document, the following symbols are used to highlight important information:

Symbol Description

Information Note:
Additional information about the current topic or procedure.

Important Note:
Information critical to the success of the procedure or use of the product.

4.2 Changes to Previous Version

• Disclaimer of License updated

4.3 Ordering Information

Roche Applied Science offers a large selection of enzymes, reagents, and systems for PCR and RT-PCR assays. For a complete overview of our products and for more detailed information on PCR and RT-PCR please visit and bookmark our Amplification Special Interest Site at http://www.roche-applied-science.com/PCR.

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transcriptor Reverse</td>
<td>250 U</td>
<td>03 531 317 001</td>
</tr>
<tr>
<td>Transcriptase</td>
<td>500 U</td>
<td>03 531 295 001</td>
</tr>
<tr>
<td></td>
<td>2,000 U</td>
<td>03 531 267 001</td>
</tr>
<tr>
<td>(4 × 500 U)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse Transcriptase</td>
<td>500 U</td>
<td>11 062 603 001</td>
</tr>
<tr>
<td>M-MuLV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In vitro transcription

Other RNases

- cDNA Synthesis System 10 reactions 11 117 631 001
- SP6 RNA Polymerase 1,000 U 10 810 274 001
- 5,000 U 10 478 671 001
- T7 RNA Polymerase 1,000 U 10 881 767 001
- 5,000 U 10 881 775 001
- T3 RNA Polymerase 1,000 U 11 031 163 001
- 5,000 U 11 031 171 001
- SP6/T7 Transcription Kit 1 kit 10 999 644 001
- DIG RNA Labeling Kit 2 × 10 reactions 11 175 025 910
- RNase 500 mg 10 109 134 001
- RNase, DNase-free 500 µg 11 119 915 001
- RNase A 25 mg 10 109 142 001
- 100 mg 10 109 169 001
- RNase H 100 U 10 786 357 001
- RNase T1 100,000 U 10 109 193 001

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