

Protector RNase Inhibitor

Cat. No. 03 335 399 001 2 000 U
Cat. No. 03 335 402 001 10 000 U (5 vials for 2 000 U)

Version Dec. 2005
Store at -15 to -25°C

Special Quality for Molecular Biology

1. What this Product Does

Contents

Vial	Content
Protector RNase Inhibitor (40 U/ μ l)	• 50 μ l (2.000 U pack size) • 5 vials of 50 μ l (10.000 U pack size) Storage buffer: 20 mM Hepes-KOH, 50 mM KCl, 8 mM dithiothreitol, 50% glycerol (v/v), pH approx. 7.6 (at 4°C)

Storage and Stability

Stable at -15 to -25°C through 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	Products*
RT-PCR	• Transcriptor Reverse Transcriptase, when used with <ul style="list-style-type: none">• Taq DNA Polymerase• FastStart Taq DNA Polymerase• Expand High Fidelity PCR System
cDNA synthesis	cDNA Synthesis System
real-time qPCR	LightCycler reagents and kits
<i>in vitro</i> transcription/ translation	T7 RNA Polymerase (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.

One-step RT-PCR	5 - 10 U
Two-step RT-PCR	25 - 50 U
<i>in vitro</i> transcription	20 U

⚠ 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 approx. 7.6 (at 4°C)

Inactivation

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

Product characteristics

Volume activity	40 U/ μ l
Molecular weight	approx. 50 kD
Purity (SDS-PAGE)	> 95%; only one visible product band
Bioburden	< 50 cfu/ml
DNA content	< 100 pg/mg
Active pH range	pH 5.0 - pH 9.0
Isoelectric point	pH 4.5
Active temperature range	25°C - 55°C (enzyme retains partial activity at 60°C)

Unit assay

One unit of Protector RNase Inhibitor is defined as the amount of protein required to inhibit 50% of the activity of 5 ng 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 μ g RNase A.

References

- Blackburn, P. (1979) Ribonuclease inhibitor from human placenta: rapid purification and assay. *J. Biol. Chem.* **254**, 12484-12493.
- Protector RNase Inhibitor Enhance Protection of RNA Against Degradation (2002) *Biochemica* **4**, 29.
- Finnegan et al (2004). Detection and strain differentiation of European bat lyssaviruses using in situ hybridisation. *Journal of Virological Methods* **121**, 223-229.
- Swayze et al. (2004) Modulation of dopamine mediated phosphorylation of AMPA receptors by PSD-95 and AKAP79/150. *Neuropharmacology* **47** 764-778.

Quality Control

Each lot of Protector RNase Inhibitor is function tested with the Titan One Tube RT-PCR Kit* and the LightCycler DPD Kit*. 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 *Eco* RI/*Hind* III*-fragments of lambda DNA is incubated with Protector RNase Inhibitor in 50 µl test buffer at 37°C for 1 h. The amount of inhibitor that causes no alteration in the banding pattern is stated under "Endo."

Absence of nicking activity: 1 µg supercoiled pBR322 DNA is incubated with Protector RNase Inhibitor in 50 µl test buffer at 37°C for 1 h. The amount of inhibitor that causes no relaxation of supercoiled DNA is stated under "Nick. Act."



Absence of ribonuclease (1): 5 µg of MS2 RNA is incubated with Protector RNase Inhibitor for 1 h at 37°C in a final volume of 50 µl. The amount of inhibitor that causes no degradation of MS2 RNA is stated under "RNase 1."

Absence of ribonuclease (2): 5 µg of MS2 RNA is incubated with Protector RNase Inhibitor for 1 h at 37°C, then 10 min at 65°C in a final volume of 50 µl. The amount of inhibitor that causes no degradation of MS2 RNA is stated under "RNase 2."

4. Supplementary Information

Symbols

In this Instruction Manual, 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.

4.1 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>

If you want to use Protector RNase Inhibitor in a production procedure or have Protector RNase Inhibitor included in a kit, you can obtain a version of this product that is produced under stringent GMP conditions. For additional details, please see the listing for RNase Inhibitor, Industrial GMP-grade, Cat. No. 3 247 058 103, in the industrial catalog, "Enzymes and Nucleotides for the Diagnostic Industry."

	Product	Pack Size	Cat. No.
RT-PCR	Transcriptor Reverse Transcriptase	250 U	03 531 317 001
		500 U	03 531 295 001
		2,000 U	03 531 287 001
		(4 × 500 U)	
	Reverse Transcriptase M-MuLV	500 U	11 062 603 001
	Expand Reverse Transcriptase	1,000 U	11 785 826 001
		5,000 U	11 785 834 001
	(not for sale in the US)		

Product	Pack Size	Cat. No.	
<i>C. therm</i> Polymerase	300 U	12 016 311 001	
<i>C. therm</i> Polymerase One-Step RT-PCR System	50 reaction	12 016 338 001	
	250 reaction	12 016 346 001	
Titan One Tube RT-PCR Kit	50 tests	11 939 823 001	
Titan One Tube RT-PCR System	25 reaction	11 888 382 001	
	100 reaction	11 855 476 001	
Transcriptor First Strand cDNA Synthesis Kit	1 kit (50 reactions)	04379012001	
First strand cDNA Synthesis Kit for RT-PCR	30 reaction	11 483 188 001	
cDNA Synthesis System	10 react.	11 117 831 001	
In vitro transcription	SP6 RNA Polymerase	1,000 U	10 810 274 001
		5,000 U	11 487 671 001
	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 reaction	11 175 025 910	
Other RNases	RNase	100 mg	10 109 126 001
		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	25 U	10 786 349 001
		100 U	10 786 357 001
	RNase T1	100,000 U	10 109 193 001
		500,000 U	10 109 207 001

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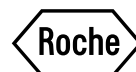
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