RNase Inhibitor, 40 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0400901	2500 U (125 rnx)	62.5 µl RNase Inhibitor
BR0400902	10000 U (500 rxn)	4 × 62.5 µl RNase Inhibitor

COMPONENT	COMPOSITION
RNase Inhibitor	RNase Inhibitor, 40 U/µl, in storage buffer containing 50% (v/v) glycerol.
STORAGE	-20°C (until expiry date – see product label)

FEATURES

- Exceptionally pure proprietary Ribonuclease Inhibitor for demanding RNA applications
- Active under variety of reaction conditions used for work with RNA
- Prevention of RNA from degradation by a wide range of RNases

APPLICATIONS

- In vitro transcription/translation
- cDNA synthesis
- RNA purification and storage

DESCRIPTION

biotechrabbit™ RNase Inhibitor is an acidic protein that is a potent inhibitor of a wide spectrum of ribonucleases. The RNase Inhibitor helps to prevent RNA degradation in applications like cDNA synthesis, RT-PCR, in vitro transcription/ translation reactions or RNA purification. The enzyme is purified from a recombinant *E. coli* strain carrying the RNase Inhibitor gene.

PROTOCOL

General guidelines

- RNase Inhibitor can be used in all common reactions performed with RNA, it is active in all common buffers.
- The recommended final concentration for RNA protection is about 1-2 units of RNase Inhibitor for every 1 µl of the reaction mixture.
- Optimal temperature for Ribonuclease Inhibitor activity is 37°C
- Inactivation conditions: 65°C for 20 minutes

Prevention of cDNA synthesis reaction contamination

RNase contamination is an exceptional concern when working with RNA. RNase A, providing most threat to RNA integrity, is a highly stable contaminant of any laboratory. To prevent RNA from degradation and to minimize possibility of contamination during cDNA synthesis; follow the guidelines below:

- Use separate clean areas for preparation of the samples and the reaction mixture.
- DEPC-treat all tubes and pipette tips or use certified nuclease-free labware with aerosol filters.
- Wear fresh gloves when handling RNA and all reagents.
- Always assess the integrity of RNA prior to cDNA synthesis in denaturing agarose gel electrophoresis.
- Use RNase free water and other reagents.
- To prevent RNA from degradation, add Ribonuclease inhibitor (optional) in to the cDNA synthesis reaction (20 units for 20 µl reaction).

Typical cDNA synthesis reaction set up

- Thaw on ice and mix very well all reagents.
- Assemble and keep all reactions on ice.
- To use time and reagents effectively, always prepare master mix for multiple reactions. For a master mix volume, always calculate the number reactions that you need plus one additional.
- Combine the following in an RNase-free reaction tube:

COMPONENT	VOLUME	FINAL CONCENTRATION
dNTP Mix (10 mM each dNTP)	2 µl	1 mM (each dNTP)
RNase Inhibitor, 40 U/µl	0.5 µl	1 U/µl
Oligo (dT)$_{12-18}$ (10 µM) – or	*0.5 µl*	*0.25 µM*
Hexamer Primer (25 µM) – or	*1 µl*	*1.25 µM*
Gene Specific Primer (10 µM)	*0.5 µl*	*0.25 µM*
RNA Template	0.1–1 µg total RNA or 50–500 ng mRNA (polyA)	
Reverse Transcriptase, 200 U/µl (i.e. BR0400301)	1 µl	10 U/µl
5× RT Buffer	4 µl	1×
RNase-free water (i.e. BR1900301)	Variable	
Total volume	20 µl	

- Mix and collect the drops by centrifuging briefly.
- When using
  - Hexamer Primer, incubate 10 minutes at 30°C followed by 50–55°C for 20–60 minutes
  - Oligo (dT) or gene-specific Primer incubate at 50–55°C for 20–60 minutes.
- Inactivate enzymes at 99°C for 5 minutes.
- Collect the drops by spinning briefly.
- Store products at –20°C or proceed to next step, like PCR or qPCR.
- *Use maximum 10 µl of the cDNA synthesis reaction mix for PCR in 50 µl volume.*

CERTIFICATE OF ANALYSIS

Unit Definition

One unit is defined as the amount of enzyme required to inhibit 50% of the activity of 5 ng RNase A (hydrolysis of cyclic cytidine-monophosphoric).

Quality Control

Protein Purity

The Protein purity is analyzed by SDS polyacrylamide gel electrophoresis.

RNase Assay

A sample of the enzyme was incubated with a RNA template. RNase activity was not observed after agarose gel electrophoresis.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

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