MB084_IFU_EN_V2401

NZY Ribonuclease Inhibitor

Catalogue number	Presentation
MB08401	2500 U
MB08402	5 x 2500 U

Description

NZY Ribonuclease Inhibitor is a recombinant protein purified from *Escherichia coli*. It inhibits the activity of ribonucleases (RNases; EC 3.1) of the pancreatic type, such as RNase A, RNase B and RNase C, by binding them noncovalently in a 1:1 ratio. NZY Ribonuclease Inhibitor is useful in any application where RNase contamination is a potential problem. For instance, it can be used to protect template RNA in cDNA synthesis reactions, RT-PCR or in vitro transcription/translation, as well as to protect viral RNA during in vitro replication. In addition, it will inhibit RNases during RNA isolation and purification and during RNase-free antibodies preparation. NZY Ribonuclease Inhibitor is not active against RNase 1, RNase T1, RNase T2, S1 nuclease and RNase H.

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store all components at -85 to -15 °C in a constant temperature freezer. Stored as specified, the product will remain stable until the expiry date.

Components

	MB08401 (2500 U)		MB08502 (5 x 2500 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
NZY Ribonuclease Inhibitor	1	65 µL	5	65 µL

Specifications

Unit Definition: One unit is defined as the amount that inhibits 50% of the activity of 5 ng RNase A. This activity is determined by measuring the inhibition of hydrolysis of cytidine 2',3'-cyclic monophosphate by RNase A.

Enzyme concentration: 40 U/µL.

Inhibition & Inactivation: NZY Ribonuclease Inhibitor is inhibited by common denaturants such as SDS, urea and all oxidizing reagents. Temperatures above 65 °C also inactivate the inhibitor. There is some residual activity up to 50-55 °C.

Standard Protocol

Recommendations before starting

Reagents usage: NZY Ribonuclease Inhibitor requires 0.5 to 1 mM DTT (not provided) in the reaction system to maintain activity. The storage buffer of this protein contains 8 mM DTT, but additional DTT is required if volume of the inhibitor in the reaction mixture is less than 1/8 of the total volume.

Procedure

NZY Ribonuclease Inhibitor can be added directly to the reaction mixtures when the RNases A, B or C could cause RNA degradation. Factors like incubation temperature, concentration of protein, buffer composition, DTT concentration and presence of stabilizing agents may affect the effective unit activity of NZY Ribonuclease Inhibitor.

For First-strand cDNA synthesis: Use 40 units of protein in a 20 µL reaction mixture to protect the template RNA, improve total cDNA yields and increase the percentage of full-length cDNA. The presence of NZY Ribonuclease Inhibitor does not affect the use of RNase H after first-strand cDNA synthesis.

For RT-PCR: Use 40 units of protein in a 20 µL reaction mixture. NZY Ribonuclease Inhibitor does not affect the enzymes used in RT-PCR.

For *In Vitro* Transcription: Use 20-40 units of protein in a 10 µL reaction mixture. NZY Ribonuclease Inhibitor is compatible with T3, T7, and SP6 RNA Polymerases.

Quality control

Purity

NZY Ribonuclease Inhibitor is >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time qPCR.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 DNA are incubated with 40 U of NZY Ribonuclease Inhibitor for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 40 U of the protein for 1 h at 37°C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids. Similar tests are performed with reaction buffer.

Functional assay

NZY Ribonuclease Inhibitor is tested in a reaction to protect the integrity of 125 ng of RNA exposed to a complex mixture of RNases from serum origin. Different amounts of NZY Ribonuclease Inhibitor are assayed in a 20 µL reaction. The integrity of RNA is judged through a real-time one-step RT-qPCR experiment. Complete preservation of RNA integrity is observed in the presence of NZY Ribonuclease Inhibitor (in all units tested) when using 1 mM DTT as cofactor, as measured by the successful amplification of the desired target in the real-time RT-PCR assay (the signal overlaps to that emitted by an equivalent RNA sample not exposed to the RNases mixture).

Troubleshooting

Troubleshooting is often a systematic, meticulous process where varying one parameter at a time and evaluating impacts can unveil the root cause of issues. These adjusted suggestions, incorporating a blend of specificity and exploratory approaches, aim to enhance the clarity and actionability of your troubleshooting guide. Should any other technical or procedural aspects require attention, your feedback and additional information will always be welcomed.

PROTEIN NOT SHOWING RNASE INHIBITION ACTIVITY
• **Presence of other RNases type**
It is possible that other types of RNases, against which the NZY Ribonuclease Inhibitor is not effective, are present as contaminants in the reaction.
• **Inadequate DTT concentration**
Ensure that an adequate amount of DTT is present in the reaction. NZY Ribonuclease Inhibitor requires a concentration of 0.5 to 1 mM DTT to maintain its activity. In addition, ensure that the DTT used is of high quality and has not degraded. DTT can degrade over time, especially when exposed to air or light. Consider preparing a fresh DTT solution if there are concerns about its quality.
• **Denaturing conditions**
Check for the presence of potential inhibitors of the NZY Ribonuclease Inhibitor. Adjust the temperature to optimize its functionality under the specified conditions.
• **Inadequate storage conditions**
Verify that the NZY Ribonuclease Inhibitor is stored properly, preferably at -15 °C or below, to prevent degradation. Since the protein contains DTT in its storage buffer ensure proper sealing to maintain activity.