

Lyo NZY Ribonuclease Inhibitor

Catalogue number:

MB41102 20,000 U
MB41103 5 x 20,000 U

Description

Lyo NZY Ribonuclease Inhibitor is a freeze-dried enzyme preparation provided with an optimized reconstitution buffer designed to confer maximal levels of stability and enzymatic activity. The enzyme is subjected to rigorous purification protocols to ensure optimal performance. Lyo NZY Ribonuclease Inhibitor 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 conditions

The product can be shipped in a range of temperatures from dry ice to room-temperature.

Storage conditions

Upon arrival, the Lyo NZY Ribonuclease Inhibitor should be stored at -20 °C. Once resuspended, the enzyme should be stored at -20 °C in a freezer without defrost cycles. The protein will remain stable till the expiry date if stored as specified.

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.

Preparation of Lyo NZY Ribonuclease Inhibitor

Reconstitute the Lyo NZY Ribonuclease Inhibitor with 500 µL of Lyo NZY-RI resuspension buffer provided. Flick the tube to mix well or pipet gently up and down; wait 2-3 min. Complete resuspension can take some time. Spin down to collect the solution. Do not replace the resuspension buffer with water or any other buffer.

Enzyme concentration

40 U/µL

Protocol

Lyo NZY Ribonuclease Inhibitor can be added directly to the reaction mixtures when the RNases A, B or C could cause RNA degradation. When preparing a reaction, make sure to add it before other components that are possible sources of RNases contamination.

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 to increase the percentage of full-length cDNA. The presence of Lyo 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. Lyo 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. Lyo NZY Ribonuclease Inhibitor is compatible with T3, T7, and SP6 RNA Polymerases.

Quality control assays

Purity

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

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 40 U of Lyo NZY Ribonuclease Inhibitor for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 40 U of Lyo NZY Ribonuclease Inhibitor for 1 hour 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.

Functional assay

Lyo 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 Lyo 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 Lyo 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).

Related products

Product name	Cat. No.
Lyo NZY Reverse Transcriptase	MB409
DTT (Dithiothreitol)	MB03101
NZY Total RNA Isolation kit	MB13402
NZY Viral RNA Isolation kit	MB40701

Troubleshooting

Protein not showing RNase inhibition activity

- Type of RNases present in the reaction

RNases for which Lyo NZY Ribonuclease Inhibitor has no activity may be present as contaminants in the reaction.

- Concentration of DTT

Check if enough amount of DTT is present in the reaction. Lyo NZY Ribonuclease Inhibitor requires 0.5 to 1 mM DTT to maintain activity.

- Denaturing conditions

Lyo 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.

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assays	Pass
Functional assay	Pass

Approved by:



Patrícia Ponte
Senior Manager, Quality Systems

For research use only.

