NZY Ribonuclease Inhibitor (no DTT)

Catalogue number:

MB41001, 2500 U (40 U/µL)
MB41002, 5 x 2500 U (40 U/µL)

Description

NZY Ribonuclease Inhibitor (no DTT) is a highly pure recombinant enzyme displaying improved resistance to oxidation, which allows it to be stable at DTT concentrations lower than 1 mM. This property makes this ribonuclease inhibitor ideal for reactions that do not tolerate higher levels of DTT (>1 mM) and were RNases (EC 3.1) contamination is a potential problem, such as in real-time RT-PCR assays. NZY Ribonuclease Inhibitor (no DTT) inhibits the activity of the omnipresent RNases of the pancreatic type, such as RNase A, RNase B and RNase C, by binding them noncovalently in a 1:1 ratio. It is not active against RNase 1, RNase T1, RNase T2, S1 nuclease and RNase H.

NZY Ribonuclease Inhibitor (no DTT) can be useful in the following applications: cDNA synthesis, RT-PCR, *in vitro* transcription, *in vitro* replication, RNA labelling or RNA isolation and purification.

Storage conditions

NZY Ribonuclease Inhibitor (no DTT) should be stored at -20 °C in a constant temperature freezer. 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.

Enzyme concentration

40 U/µL

Protocol

NZY Ribonuclease Inhibitor (no DTT) 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. Generally, the recommended concentration of NZY Ribonuclease Inhibitor (no DTT) in a reaction is 1 unit/µL. For specific applications, the optimal concentration can be determined by titrating the enzyme in the reaction.

Quality control assays

Purity

NZY Ribonuclease Inhibitor (no DTT) 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 NZY Ribonuclease Inhibitor (no DTT) for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 40 U of NZY Ribonuclease Inhibitor (no DTT) 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

NZY Ribonuclease Inhibitor (no DTT) 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 (no DTT) 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 (no DTT) (in all units tested), 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.
NZY Reverse Transcriptase	MB124
NZY Total RNA Isolation kit	MB13402
NZY Viral RNA Isolation kit	MB40701
Water for Molecular Biology	MB11101

Troubleshooting

Protein not showing RNase inhibition activity
• Type of RNases present in the reaction
RNases for which NZY Ribonuclease Inhibitor (no DTT) has no activity may be present as contaminants in the reaction.
• Denaturing conditions
NZY Ribonuclease Inhibitor (no DTT) is inhibited by common denaturants such as SDS and urea. Temperatures above 65 °C also inactivate the inhibitor. There is significant 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

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