MB410_IFU_EN_V2401

# NZY Ribonuclease Inhibitor (no DTT)

| Catalogue number | Presentation |
|---|---|
| MB41001 | 2500 U |
| MB41002 | 5 x 2500 U |

## Description

NZY Ribonuclease Inhibitor (no DTT) is a 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.

## 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

| | MB41001 (2500 U) | | MB41002 (5 x 2500 U) | |
|---|---|---|---|---|
| **COMPONENT** | **TUBES** | **VOLUME** | **TUBES** | **VOLUME** |
| NZY Ribonuclease Inhibitor (no DTT) | 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 (no DTT) 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 (no DTT) does not requires addition of extra DTT in reactions.

**Procedure**

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

**Purity**

NZY Ribonuclease Inhibitor (no DTT) 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 (no DTT) 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 (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).

## 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. |
| • **Denaturing conditions** |
| Check for the presence of potential inhibitors of the NZY Ribonuclease Inhibitor (no DTT). Adjust the temperature to optimize its functionality under the specified conditions. |
| • **Inadequate storage conditions** |
| Verify that the NZY Ribonuclease Inhibitor (no DTT) is stored properly, preferably at -15 °C or below, to prevent degradation. Since the protein contains some DTT in its storage buffer ensure proper sealing to maintain activity. |