# NZY Ribonuclease Inhibitor

**Catalogue number:**

MB08401, 2500 U (40 U/µL)
MB08402, 5 × 2500 U (40 U/µL)

## 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.

## Storage conditions

NZY Ribonuclease Inhibitor 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 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.

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.

**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 assays

### Purity

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 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 NZY Ribonuclease Inhibitor during 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 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).

## Related products

| Product name | Cat. No. |
|---|---|
| NZY Reverse Transcriptase | MB124 |
| DTT (Dithiothreitol) | MB03101 |
| NZY Total RNA Isolation kit | MB13402 |
| 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 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. NZY Ribonuclease Inhibitor requires 0.5 to 1 mM DTT to maintain activity. |
| • Denaturing conditions |
| 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:

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