MB412_IFU_EN_V2501

Lyo NZY Ribonuclease Inhibitor (no DTT)

Catalogue number	Presentation
MB41204	For 105 µL (100 rxns of 20 µL)
MB41205	For 5 x 105 µL (500 rxns of 20 µL)

Description

Lyo NZY Ribonuclease Inhibitor (no DTT) 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 and displays 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. Lyo 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. This enzyme is not active against RNase 1, RNase T1, RNase T2, S1 nuclease and RNase H. Lyo NZY Ribonuclease Inhibitor (no DTT) may be applied 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 can be shipped at a range of temperatures from dry ice to room temperature. Upon receipt, store all components at -85 to -15 °C in a constant temperature freezer. The protein will remain stable until the expiry date if stored as specified. Once resuspended, the enzyme should be stored in the same conditions previously mentioned.

Components

COMPONENT		MB41204 (100 rxns)		MB41205 (500 rxns)	
		TUBES	VOLUME	TUBES	VOLUME
Lyo NZY RI (no DTT) 40 U/µL	Lyo NZY Ribonuclease Inhibitor (no DTT) 40 U/µL	1	for 105 µL	5	for 105 µL
RBuffer for Lyo NZY RI (no DTT)	Reconstitution buffer for Lyo NZY Ribonuclease Inhibitor (no DTT)	1	200 µL	5	200 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: 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.

Standard Protocol

Recommendations before starting

Reagents usage: Lyo NZY Ribonuclease Inhibitor (no DTT) does not requires addition of extra DTT in reactions.

Procedures before starting

Reconstitute the Lyo NZY RI (no DTT) 40 U/µL as follows:

a. Add **105** µL of the provided Reconstitution buffer for Lyo NZY Ribonuclease Inhibitor (no DTT) **– RBuffer for Lyo NZY RI (no DTT) –** directly into the tube containing the lyophilized protein.
   Note: *Do not replace the resuspension buffer with water or any other buffer.*
b. Flick the tube to mix thoroughly or pipette gently up and down;
c. Place the tube on ice and allow the reconstitution process to take place for 2-3 min. Complete resuspension can take some time.
d. Spin down to collect the solution.

Procedure

Upon resuspension, Lyo NZY RI (no DTT) 40 U/µL 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 Lyo NZY RI (no DTT) 40 U/µL 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

Lyo NZY RI (no DTT) 40 U/µL 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 Lyo 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

Lyo NZY Ribonuclease Inhibitor (no DTT) is tested in a reaction to protect the integrity of 125 ng of RNA exposed to 5 ng of RNase A 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 RI (no DTT) 40 U/µL, 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
• **Protein not active**
Prior to use, ensure to reconstitute the lyophilized protein according to the provided instructions (refer to Procedures before Starting). Do not reconstitute the protein with water, or any other buffer. The enzyme should be reconstituted with the RBuffer for Lyo NZY RI (no DTT) provided, acquiring its functional activity thereafter.
• **Presence of other RNases type**
It is possible that other types of RNases, against which the Lyo NZY Ribonuclease Inhibitor (no DTT) is not effective, are present as contaminants in the reaction.
• **Denaturing conditions**
Check for the presence of potential inhibitors of the Lyo NZY Ribonuclease Inhibitor (no DTT). Adjust the temperature to optimize its functionality under the specified conditions.
• **Inadequate storage conditions**
Verify that the Lyo NZY RI (no DTT) 40 U/µL 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.