Lyo NZY Reverse Transcriptase

Catalogue number: MB40902, 100000 U

Description

Lyo NZY Reverse Transcriptase is a freeze-dried enzyme provided with an optimized reconstitution buffer designed to confer maximal levels of stability and enzymatic activity. Lyo NZY Reverse Transcriptase is a modified recombinant form of the Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase purified from *Escherichia coli*. Lyo NZY Reverse Transcriptase synthesizes the complementary DNA strand in the presence of a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template in a wide range of temperatures (37-50°C). High reaction temperatures are beneficial to improve specificity and to allow synthesis of cDNA from complex templates, including those with high GC-content or with high degree of secondary structure. The enzyme lacks 3´→5´ exonuclease activity and has no RNase H activity, enabling improved synthesis of full-length cDNA even for long mRNA, using random priming. Thus, Lyo NZY Reverse Transcriptase gives high yields of cDNA up to 7 kb. Lyo NZY Reverse Transcriptase can be used in first-strand cDNA synthesis experiments, RT-PCR, RT-qPCR, DNA labelling and analysis of RNA by primer extension.

Shipping conditions

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

Storage conditions

Upon arrival, Lyo NZY Reverse Transcriptase 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.

System components

Component	MB40901, 100000 U
Lyo NZY Reverse Transcriptase	1 tube with lyophilized enzyme
Lyo NZY-RT resuspension buffer	0.6 mL
10x Reaction buffer for Lyo NZY-RT	1.2 mL
0.1 M DTT	0.6 mL

Unit definition

One unit is defined as the amount of enzyme necessary to catalyse the incorporation of 1 nmol of dTTP into acid-insoluble material in 10 min at 37 °C, using poly(A)×oligo(dT)$_{12\text{-}18}$ as a template-primer.

Preparation of Lyo NZY Reverse Transcriptase

Reconstitute the Lyo NZY Reverse Transcriptase with 500 µL of Lyo NZY-RT resuspension buffer. 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: 200 U/µL after resuspension.

Inhibition and Inactivation

Lyo NZY Reverse Transcriptase is inhibited in the presence of metal chelators (e.g. EDTA), inorganic phosphate, pyrophosphate and polyamines. The enzyme is inactivated at 98 °C for 5 min.

Protocol for first-strand cDNA synthesis

Since RNA is very susceptible to degradation, a correct handling and storage of this nucleic acid is essential. Special precautions should be taken to avoid RNAse contamination.

1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components:

total RNA; *or* mRNA / poly(A) RNA	10 pg – 5 µg 10 pg – 0.5 µg
Oligo(dT)$_{12\text{-}18}$ (50-60 µM); *or* random hexamer (50-250 ng/µL); *or* gene-specific primer (2 µM)	1 µL (2.5-3 µM final conc.); (*or* 2.5-12.5 ng/µL final conc.); (*or* 0.1 µM final conc.)
dNTP Mix (10 mM each)	1 µL (0.5 mM final conc.)
Nuclease-free water	up to 16 µL

2. For some GC-rich RNAs or nucleic acids with high degree of secondary structure, a denaturation step may be required. If so, centrifuge briefly and incubate the mix at 65 °C for 5 min. Chill on ice for at least 1 min, briefly centrifuge again and place on ice.

3. Add the following reaction components:

10x Reaction buffer for Lyo NZY-RT (*)	2 µL
0.1 M DTT	1 µL
NZY Ribonuclease Inhibitor (not provided) (**)	1 µL (40 units)
Lyo NZY Reverse Transcriptase	1 µL (200 units)
FINAL Volume	**20 µL**

(*) Does not contain DTT. Enzyme requires 5 mM for optimum activity. NZYTech provides 0.1 M DTT separately.
(**) NZY Ribonuclease Inhibitor (Cat. No. MB084) will protect RNA, improve total cDNA yields and increase the percentage of full-length cDNA. Its addition is indispensable when amount of RNA template is ≤ 80 ng.

4. Mix gently and centrifuge briefly.

5. Incubate at 50 °C for 30-50 min.

Note: *When using random-hexamer primers, incubate first at 25 °C for 10 min and then proceed with the 30-50 min reaction step (step 5).*

6. Inactivate the reaction by heating at 85 °C for 5 min, and then chill on ice.

7. Store cDNA product at -20 °C or proceed to downstream applications.

Important notes

- cDNA can be stored at -20 °C or at 4 °C for up to one week. If long-term storage is required, -70 °C is recommended. Avoid freeze/thaw cycles of the cDNA.
- The resulting cDNA can be used for cloning or as a template in PCR or qPCR reactions. Typically, 10% (2 µL) of the first-strand reaction is enough for most PCR applications. Optionally, the cDNA can be diluted in TE buffer.

- When using cDNA in PCR amplification, some targets (> 1 kb) may require RNA-free DNA as template. To remove RNA bond to cDNA, add 1 µL (5 U) of NZY RNase H (Cat. No. MB085) and incubate at 37 °C for 20 min. This procedure will increase the sensitivity of the PCR step.

Quality control assays

Purity

Lyo NZY Reverse Transcriptase 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 DNA are incubated with 200 U of Lyo NZY Reverse Transcriptase for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 200 U of the enzyme 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. Other components are also screened for the presence of contaminating nucleases.

Functional assay

Lyo NZY Reverse Transcriptase and respective buffer are tested for performance in a RT-qPCR experiment: a 10-fold serial dilution of total RNA from mouse liver (1 µg to 0.1 ng) is reverse transcribed using 200 units of enzyme; the resultant cDNA is then used as template in a quantitative real-time PCR assay using specific primers to amplify the mouse GAPDH gene.

Troubleshooting

Little or no RT-PCR/RT-qPCR amplification product
• RNA damage or degradation
Analyse RNA on a denaturing gel to verify integrity. Use aseptic conditions while working with RNA to prevent RNase contamination. Ensure the use of NZY Ribonuclease Inhibitor; the addition of this inhibitor is essential when using less than 50 ng of RNA in order to safeguard the template against degradation due to ribonuclease contamination. Replace RNA if necessary.
• Presence of RT inhibitors
Some inhibitors of RT enzymes include: SDS, EDTA, glycerol, sodium phosphate, spermidine, formamide and guanidine salts. They can be problematic in smaller reaction volumes. If necessary, remove inhibitors by ethanol precipitation of the RNA preparation before use; wash the pellet with 70% (v/v) ethanol.
• Not enough starting RNA
Increase the concentration of starting RNA.
Unexpected bands after electrophoretic analysis of amplified products
• Genomic DNA contamination
DNase I may be used to eliminate genomic DNA contamination from the starting RNA (pre-treatment RNA). The enzyme volume should not exceed 10% of the total reaction volume.

V2003

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assays	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
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