NZY M-MuLV Reverse Transcriptase

Catalogue number: MB08301, 20,000 U
MB08302, 100,000 U

Description

NZY M-MuLV Reverse Transcriptase is a recombinant form of the Reverse Transcriptase from the Moloney Murine Leukemia Virus (M-MuLV) purified from *Escherichia coli*. The enzyme synthesizes the complementary DNA strand in the presence of a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template. NZY M-MuLV Reverse Transcriptase 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, the enzyme gives high yields of first-strand cDNA up to 7 kb. NZY M-MuLV 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

NZY M-MuLV Reverse Transcriptase is shipped on dry ice.

Storage conditions

NZY M-MuLV Reverse Transcriptase should be stored at -20 °C in a freezer without defrost cycles. The protein will remain stable up to 3 years if stored as specified.

Storage buffer

20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) Triton X-100, 50% (v/v) glycerol.

Reaction buffer (10×)

500 mM Tris-HCl, pH 8.3, 750 mM KCl, 30 mM $MgCl_2$, 100 mM DTT. Upon thawing, if any precipitate is observed, pulse vortex until the precipitate is completely resuspended

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$(dT)_{12-18}$ as a template-primer.

Enzyme concentration: 200 U/µL

Inhibition and Inactivation

NZY M-MuLV Reverse Transcriptase is inhibited in the presence of metal chelators (e.g. EDTA), inorganic phosphate, pyrophosphate and polyamines. The enzyme is inactivated at 70 °C for 15 min.

Protocol for first-strand cDNA synthesis

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 – 500 ng
Oligo$(dT)_{12-18}$, or random hexamer, or gene-specific primer	x µL (50 µM) x µL (50 – 250 ng) x µL (2 pmol)
dNTP Mix (10 mM each)	x µL (0.5 mM final concentration)
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:

10× Reaction Buffer	2 µL
NZY Ribonuclease Inhibitor (MB084)	1 µL
NZY M-MuLV Reverse Transcriptase	1 µL (200 U)
Final Volume	20 µL

4. Mix gently and centrifuge briefly.

5. Incubate at 37 °C for 50 min.
Note: When using random-hexamer primers, incubate first at 25 °C for 10 min and then at 37 °C for 50 min.

6. Inactivate the reaction by heating at 70 °C for 15 min.

7. Store cDNA product at -20 °C or proceed to next step(s).

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 complementary to the cDNA, add 1 µL (5 units) of NZY RNase H (MB085) and incubate at 37 °C for 20 min. This procedure will increase the sensitivity of the PCR step.

Quality control assays

Purity

NZY M-MuLV Reverse Transcriptase is >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining.

Nuclease assays

To test for DNase activity, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 40 U of NZY M-MuLV Reverse Transcriptase for 14-16 hours at 37 °C. To test for RNase activity, 1 µg of RNA is incubated with 40 U of NZY M-MuLV Reverse Transcriptase 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. Similar tests are performed with the NZY M-MuLV Reverse Transcriptase reaction buffer.

Functional assay

NZY M-MuLV Reverse Transcriptase is tested for performance in a RT-PCR experiment designed to calculate the number of mRNA copies of the GAPDH gene in mouse liver cells. 200 U of NZY-MuLV Reverse Transcriptase are used to reverse transcribe 1 µg of total RNA extracted from mouse liver. Similar functional tests are performed with NZY M-MuLV Reverse Transcriptase reaction buffer.

Related products

Product name	Cat. No.
NZY Ribonuclease Inhibitor	MB084
NZY RNase H (*E. coli*)	MB085
Oligo $(dT)_{18}$ primer mix	MB12801
Random hexamer mix	MB12901
dNTPs NZYMix	MB086
NZY Reverse Transcriptase	MB124

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.
• RT primers blocked by secondary structures
The temperature increase may be required. Try the thermo-stable NZY Reverse Transcriptase (MB124) to perform the RT reaction at 50 °C instead of NZY M-MuLV Reverse Transcriptase.
• 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.

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Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assay	Pass
Functional assay	Pass

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