NZY M-MuLV Reverse Transcriptase

Catalogue number	Presentation
MB08301	20000 U
MB08302	100000 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, within an optimal temperature range of 37-42 °C. 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. NZY M-MuLV Reverse Transcriptase yields high cDNA quantities of up to 3 kb and supports reverse transcription of up to 7 kb. For long templates, enzyme amount optimization may be necessary. 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 & Storage Conditions

This product is shipped in dry ice. Upon receipt, store all components at -30 °C to -15 °C in a constant temperature freezer. Stability can be extended by storing them at -85 °C to -65 °C. These meticulous storage procedures ensure that the NZY M-MuLV Reverse Transcriptase delivers consistent and reliable results across its lifespan and usage. All components are formulated to be ready to use. The kit will remain stable till the expiry date if stored as specified.

Components

	MB08301 (20000 U)		MB08302 (100000 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
M-MuLV Reverse Transcriptase (20000 U)	1	100 µL	5	100 µL
10x Reaction buffer for Reverse Transcriptases	1	750 µL	2	750 µL

Specifications

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.

Enzyme concentration: 200 U/µL

Inhibition & 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.

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: High-quality intact RNA, free of residual genomic DNA and RNases, is essential for full-length, high-quality cDNA synthesis, and accurate RNA quantification. To ensure the integrity and purity of RNA, follow these precautions:
  - Maintain aseptic conditions: Always wear gloves, change them if suspected of contamination. We recommend using RNase-free plasticware/reagents, filtered tips and work in an RNase-free area (RNase Cleaner, Cat. No. MB16001, can help removing RNases from surfaces and materials). Designate a dedicated area and equipment solely for RNA work.
  - Store template RNA at -85 °C to -65 °C and avoid subjecting RNA to multiple freeze/thaw cycles. Perform all reaction steps on ice.
  - Assess the RNA purity concerning contaminants by examining the ratio of absorbance at 260 nm and 280 nm (A260/A280). Ideally, pure RNA should exhibit an A260/A280 ratio within the range of 1.9-2.1 in a 10 mM Tris-HCl buffer at pH 7.5.
  - DNase I (not provided) may be used to eliminate genomic DNA contamination from the starting total RNA.
- Reagent usage: The reaction buffer provided already contains Mg^{2+} at the optimal concentration for NZY M-MuLV Reverse Transcriptase activity. In extremely rare cases, the addition of extra Mg^{2+} for concentration adjustments may be considered. Upon thawing, if any precipitate is observed in the buffer, pulse vortex until the precipitate is completely resuspended. To prevent template RNA degradation, it is strongly advised to include a Ribonuclease Inhibitor in the reaction. This inhibitor protects RNA, enhances total cDNA yields, and increases

the percentage of full-length cDNA. Its inclusion is essential when the amount of RNA template is ≤ 80 ng. Please consult the NZYtech portfolio for available Ribonuclease Inhibitors.

- Handling instructions: Keep all reagents on ice while setting up the reactions. Minimize the duration of RNA exposure to ice.

Procedure for first-strand cDNA synthesis

1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components (not provided)
   Note: *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed.*

COMPONENT	1 REACTION VOLUME / AMOUNT
total RNA; or mRNA / poly(A) RNA	10 pg – 5 µg 10 pg – 0.5 µg
Oligo(dT)$_{12-18}$ (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.)
DEPC-treated water (*)	up to 16 µL

(*) Please consult the NZYtech portfolio for the components required but not provided.

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 reaction mixture at 65 °C for 5 min. Chill on ice for at least 1 min, briefly centrifuge again and place on ice.
3. On ice, perform the reverse transcription step, by adding the following reaction components to the previous mixture:

COMPONENT	1 REACTION VOLUME
10× Reaction Buffer	2 µL
NZY Ribonuclease Inhibitor (*)	1 µL (40 units)
NZY M-MuLV Reverse Transcriptase	1 µL (200 units)
FINAL VOLUME =	20 µL

(*) Please refer to the NZYtech portfolio for components required but not provided. Review the recommendations for reagent usage before starting (section above).

4. Mix gently and centrifuge briefly.
5. Incubate at 37 - 42 °C for 50 min.

Note: *When using random-hexamer primers, incubate first at 25 °C for 10 min and then proceed with the 37 - 42 °C incubation (step 5).*

6. Inactivate the reaction by heating at 70 °C for 15 min, and then chill on ice.
7. Store at - 85 °C to -15 °C or proceed to downstream applications.
   Notes
   - *cDNA can be stored frozen at -30 °C to -15 °C for short-term storage. It is also stable for up to one week when stored at 2 °C to 8 °C. For long-term storage, it is recommended to store at -85 °C to -65 °C. 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 performing qPCR using the synthesized cDNA as a template, ensure that no more than 1/10 of the final PCR volume is derived from the reverse-transcription product. For example, use up to 5 µL of cDNA obtained in the first-strand synthesis in a 50 µL PCR reaction.*

Quality control

Purity

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

Genomic DNA contamination

The product must be free of any detectable DNA contamination as evaluated through qPCR.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 DNA are incubated with 200 U of NZY M-MuLV 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. Similar tests are performed with reaction buffer.

Functional assay

NZY M-MuLV Reverse Transcriptase and respective buffer are tested for performance in a RT-qPCR experiment using a 10-fold serial dilution of total RNA from mouse liver (1 µg to 0.1 ng). The resultant cDNA is then used as template in a quantitative real-time PCR assay using specific primers to amplify mouse housekeeping genes.

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.

NO OR INSUFFICIENT AMPLIFICATION PRODUCT IN RT-PCR/RT-qPCR
• **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 to safeguard the template against degradation due to ribonuclease contamination. Replace water, plasticware and even the RNA if necessary.
• **Presence of RT inhibitors**
Some inhibitors of RT enzymes include: SDS, EDTA, glycerol, sodium phosphate, spermidine, formamide and guanidine salts. Remove inhibitors by ethanol precipitation of the RNA preparation before use; wash the pellet with 70% (v/v) ethanol. For optimal outcomes, it is advised to start with RNA that has undergone purification using a silica-based method. Check purity of RNA template by determining the A260/A280 ratio.
• **Not enough starting RNA**
Increase the concentration of starting RNA by optimizing the RNA extraction or purification process. Employ methods to enhance RNA yield or consider starting with a higher quantity of starting RNA in the reverse transcription reaction. Ensure that the RNA quality is maintained during the extraction process and reevaluate the RNA concentration using a reliable quantification method.
• **Inadequate temperature of reverse transcription**
The optimal reaction temperature for NZY M-MuLV Reverse Transcriptase activity is 37 °C. However, for high-complexity RNAs with considerable secondary structure, raising the temperature to 42 °C may be advantageous. In cases of very complex template structures, a thermostable enzyme such as NZY Reverse Transcriptase (Cat. No. MB124) may be preferable.
• **Inadequate amount of Reverse Transcriptase**
Using 1 µL of NZY M-MuLV Reverse Transcriptase is a typical starting point for standard first-strand cDNA synthesis. However, in specific cases, such as reverse transcribing long templates, adjusting the enzyme amount may be necessary for optimal results. We highly recommend conducting a titration of the enzyme volume to determine the optimal amount, starting with half or less than 200 units.
• **Incorrect priming**
For long templates or when the template is difficult to copy entirely, the use of random hexamers provides versatility by priming throughout the RNA's entire length, resulting in a cDNA pool of varied lengths. On the other hand, oligo(dT) primers are specific for poly(A)+-selected RNA, which encompasses most eukaryotic mRNAs. Utilizing both types of primers, by employing a mixture of random hexamers and oligo(dT) primers, can improve cDNA yields in certain circumstances.
• **Problems related to the PCR/qPCR setup**
Lack of product or delayed product detection in real-time PCR may arise from issues during cDNA amplification rather than during reverse transcription. To address these challenges, consider the following: - Primer Design and Concentration: Confirm that primer design adheres to best practices. Optimize primer concentrations for efficient amplification.

- Degradation of Primers/Probe: Store primers/probe appropriately to prevent degradation. Use fresh, high-quality primers/probes for each experiment.
- PCR Temperature and Cycling Conditions: Ensure that PCR temperature profiles and cycling conditions are optimal for the assay. Validate and, if necessary, optimize the annealing and extension temperatures.
- Insufficient Starting Template: Increase the concentration of the starting cDNA template if necessary. Reassess the RNA input to ensure adequate cDNA synthesis.
- Pipetting Errors: Double-check pipetting accuracy to avoid errors in reagent volumes. Use calibrated pipettes for precision in dispensing reagents.
- PCR Enzyme/Master Mix: Verify the integrity and activity of the PCR enzyme/master mix. Consider using a fresh aliquot or a different batch if there are concerns about the quality.
- Detection step: Ensure that fluorescence detection occurs during the extension step of the real-time PCR cycling program. Verify that the correct fluorescent channel is being used.

UNEXPECTED BANDS AFTER ELECTROPHORETIC ANALYSIS OF AMPLIFIED PRODUCTS / MULTIPLE PEAKS IN THE MELTING CURVE

- **Non-specific Amplification**

In the reverse transcription step, optimize reverse transcription conditions and priming. Consider using gene-specific primers or the highly thermostable Supreme NZY Reverse Transcriptase (Cat. No. MB448). For the PCR/real-time PCR step, implement a hot start PCR strategy or use an enzyme designed to minimize nonspecific amplification during the initial stages. Additionally, optimize primer design, and verify primer specificity through bioinformatics tools. Adjust annealing temperatures accordingly.

- **Contaminated Reagents or Equipment**

Use sterile and filtered tips, fresh reagents, and regularly clean pipettes and equipment. Use molecular-grade water and DEPC-treated water.

AMPLIFICATION IN THE NTC

- **Genomic DNA contamination**

Include a control PCR reaction without NZY M-MuLV Reverse Transcriptase (no RT control) to assess the presence of genomic DNA contamination; this control will also help to confirm the specificity of the primers for cDNA amplification. Design primers that span exon-exon junctions to ensure specificity for cDNA amplification. If required, apply DNase I treatment to the RNA samples before reverse transcription to eliminate any residual genomic DNA, by ensuring the DNase I treatment is thorough and follows the recommended protocol. Additionally, the cDNA template can be diluted before being used in the PCR to decrease the chances of genomic DNA contamination, as genomic DNA is present in much higher amounts than cDNA.