NZY RT SuperMix 5x

Catalogue number	Presentation
MB51001	200 µL (50 rxns of 20 µL)
MB51002	5 x 200 µL (250 rxns of 20 µL)

Features

- **Fast cDNA Synthesis**: Enables efficient first-strand cDNA synthesis in as fast as 10 minutes, reducing reaction time without compromising yield or quality.
- **Reliable Performance:** Designed to generate consistent amounts of cDNA, even from low RNA inputs, supporting downstream applications with high accuracy and sensitivity.
- **Optimal reaction conditions:** Operates effectively at 50–55 °C, promoting the denaturation of complex RNA secondary structures and enhancing transcription efficiency.
- **Convenient ready-to-use format:** Provided as a ready-to-use 5x master mix, this formulation includes all components necessary for cDNA synthesis (except the RNA template), minimizing preparation steps and reducing potential errors.
- **Broad application range:** Ideal for a variety of reverse transcription assays, including real-time PCR, RNA quantification, and gene expression studies.

Description

NZY RT SuperMix 5x is a ready-to-use next-generation master mix designed for fast and efficient first-strand cDNA synthesis. Its 5x concentrated format simplifies experimental workflows while ensuring high sensitivity and precision, even with low-input RNA or challenging sample types.

At the heart of the mix is NZY Reverse Transcriptase (RNase H minus), paired with NZY Ribonuclease Inhibitor, which safeguards RNA integrity by preventing degradation from contaminating RNases throughout the reaction. The mix contains all essential components for robust cDNA synthesis—excluding the RNA template—including dNTPs, an optimized RT buffer, and a dual-priming system. This priming strategy combines random hexamers for broad template coverage and oligo(dT)$_{18}$ primers for selective poly(A)+ mRNA targeting, offering flexibility across diverse experimental designs. Thanks to its specially formulated enzymes and stabilizers, NZY RT SuperMix 5x enables cDNA synthesis in 10 minutes at 50-55°C, facilitating the melting of RNA secondary structures for efficient transcription. It supports a wide dynamic range of RNA input, from as little as 10 pg to 5 µg of total RNA, ensuring consistent performance across various sample sources and it works

Ideal for downstream applications such as real-time PCR, RNA quantification, and gene expression analysis, this advanced mix delivers reproducible, high-quality results with minimal hands-on time, setting a strong standard for easy and consistent cDNA synthesis molecular biology workflows.

Shipping & Storage Conditions

This product is shipped in blue ice. Upon receipt, store all the components provided with NZY RT SuperMix 5x at -85 °C to -15 °C in a constant temperature freezer. The SuperMix component is designed to withstand a minimum of 15 freeze-thaw cycles without significantly losing performance. These meticulous storage procedures ensure that the NZY RT SuperMix 5x delivers consistent and reliable results across its lifespan and usage. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	MB51001 (50 reactions)		MB51002 (250 reactions)	
	TUBES	VOLUME	TUBES	VOLUME
NZY RT SuperMix 5x	1	200 µL	5	200 µL
DEPC-treated H_2O	1	1 mL	1	1 mL

Standard Protocol

Recommendations before starting

Nucleic acid Handling: The synthesis of full-length, high-quality cDNA and accurate RNA quantification depends on the use of high-quality, intact RNA that is free from residual genomic DNA and RNases. To ensure RNA integrity and purity, adhere to the following best practices:

- Aseptic Conditions: Always wear gloves and replace them immediately if contamination is suspected. Use only RNase-free plasticware, reagents, and filtered tips, and work in an RNase-free environment. To eliminate RNase contamination from surfaces and materials, use RNase & DNase Cleaner (Cat. No. MB463). Dedicate a specific area and equipment exclusively for RNA work to avoid cross-contamination.
- RNA Storage: Store template RNA at -85 °C to -65 °C to preserve its integrity. Avoid multiple freeze/thaw cycles, which can degrade RNA. Perform all reaction steps on ice to prevent thermal degradation.
- RNA Purity Assessment: Verify RNA purity by measuring the absorbance ratio at 260 nm and 280 nm (A_{260}/A_{280}). Pure RNA should have an A_{260}/A_{280} ratio greater than 1.8 when measured in a 10 mM Tris-HCl buffer at pH 7.5. This ratio indicates minimal protein contamination and is crucial for reliable downstream applications.
- Genomic DNA Removal: If genomic DNA contamination is a concern, treat the RNA sample with DNase (not provided) before proceeding with cDNA synthesis. This step is particularly important when working with total RNA to ensure accurate reverse transcription.

Reagent usage: The NZY RT SuperMix 5x is formulated to be ready to use. If a precipitate is observed upon thawing the mix, briefly vortex it until the precipitate is fully dissolved.

Handling instructions: Keep all reagents on ice while setting up reactions to maintain enzyme stability and prevent RNA degradation. Minimize the time that RNA is exposed, even on ice, to reduce the risk of degradation.

Procedure for first-strand cDNA synthesis

1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components:
   Note: *If setting up more than one reaction, prepare a reaction mixture with a volume 10% greater than the total required for the number of reactions to be performed.*
   Note: *It is highly recommended to include a negative control without RNA.*

COMPONENT	1 REACTION VOLUME / AMOUNT
DEPC-treated H_2O	up to 20 µL
total RNA; or mRNA / poly(A) RNA	10 pg – 5 µg 10 pg – 0.5 µg
NZY RT SuperMix 5x	4 µL

2. Mix and briefly centrifuge the reaction mixture.
3. **(Optional)** For complex templates it might be beneficial to include an initial incubation step at 25 °C for 10 min.
4. Incubate at 50 °C for 10 min.

   Notes
   - *In NZY RT SuperMix 5x, cDNA priming is facilitated by random hexamers and/or oligo(dT)$_{18}$ primers. While this approach inherently limits the processivity of the mix compared to site-specific priming strategies, it ensures versatility for a broad range of RNA templates. Despite this, the mix efficiently extends cDNA targets up to 1 kb in length.*
5. Inactivate the reaction by heating at 85 °C for 5 min and then chill on ice.
6. **(Optional)** Add 1 µL of NZY RNase H (NZYtech, MB085) and incubate at 37 °C for 20 min.
   Note: *Addition of NZY RNase H will remove RNA bond to cDNA. This procedure is mainly recommended when using cDNA in downstream applications that either necessitate RNA-free DNA as a template or demand high sensitivity.*
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 Reverse Transcriptase and NZY Ribonuclease Inhibitor present in the NZY RT SuperMix 5x are >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Nucleases assay

All components of the NZY RT SuperMix 5x are tested for DNases and RNases contamination, using 0.2-0.3 µg of pNZY28 plasmid DNA and 1 µg of RNA, respectively. Following incubation at 37 °C, 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

The NZY RT SuperMix 5x is tested for first-strand cDNA synthesis using a 10-fold serial dilution of total RNA from mouse liver (1 µg to 10 pg). 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**
Verify RNA integrity by running a sample on a denaturing gel. Use aseptic conditions to prevent RNase contamination. Replace contaminated reagents and RNA as needed.
• **Presence of RT inhibitors**
Remove potential inhibitors (e.g., SDS, EDTA, glycerol) from the RNA preparation by ethanol precipitation, followed by washing the pellet with 70% ethanol. Start with RNA purified using a silica-based method and verify RNA purity (A_{260}/A_{280} ratio).
• **Insufficient Starting RNA**
Optimize RNA extraction to increase yield or start with a higher RNA concentration in the reverse transcription reaction. Reassess RNA quality and concentration using reliable quantification methods.
• **Short incubation time for long targets**
For longer or complex RNA templates (> 0.5 kb), extend the reverse transcription incubation time from the standard 10 minute to 15-20 minutes to enhance cDNA yield.
• **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
• **Contaminated Reagents or Equipment**
Use sterile, filtered tips, fresh reagents, and clean equipment regularly. Ensure that only molecular-grade water is used.

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