Supreme NZY First-Strand cDNA Synthesis Kit

Catalogue number	Presentation
MB50001	50 reactions
MB50002	250 reactions

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Features

- **Rapid cDNA Synthesis**: Enables efficient first-strand cDNA synthesis in just 1 minute, significantly reducing reaction time without compromising yield or quality.
- **Broad Temperature Compatibility:** Optimized for use across a wide range of temperatures (30–70°C), with a recommended reaction temperature of 60°C for enhanced efficiency and robustness.
- **Superior Performance:** Designed to deliver high yields of full-length cDNA, even with low RNA input amounts, supporting downstream applications with unmatched accuracy and sensitivity.
- **Streamlined Workflow:** Provided as a ready-to-use 5x master mix and an enzyme mix, accelerating cDNA synthesis workflow while reducing potential errors.
- **Versatile Applications:** Ideal for a variety of reverse transcription assays, including real-time PCR, RNA quantification, and gene expression studies.

Description

Supreme NZY First-Strand cDNA Kit is an all-in-one solution, engineered to deliver unmatched efficiency and speed for first-strand cDNA synthesis. This ready-to-use formulation simplifies experimental workflows while providing exceptional sensitivity and accuracy, even with challenging RNA samples or low-input quantities. The kit includes a Supreme NZY RT Enzyme Mix which contains at its core the Supreme NZY Reverse Transcriptase, a second-generation enzyme celebrated for its outstanding stability, speed, accuracy, and consistency, ensuring superior RT-qPCR data quality. Additionally, the mix incorporates the Supreme NZY Ribonuclease Inhibitor to ensure RNA integrity by protecting it from RNase contamination throughout the process. Supreme NZY RT Master Mix 5x incorporates all the remaining necessary components for effective cDNA synthesis, except the RNA template. This includes dNTPs, an optimized buffer, and a carefully balanced mixture of primers – random hexamers for comprehensive RNA template priming and oligo(dT)$_{18}$ primers for selective targeting poly(A)+ mRNA, providing flexibility to address a wide range of experimental needs.

Designed for versatility, Supreme NZY First Strand cDNA Synthesis Kit operates across a broad temperature range (30–70°C), with optimal performance at 60°C, making it suitable for diverse sample types and applications. Its proprietary enzyme blend and stabilizing components enable rapid cDNA synthesis in just 1 minute, significantly accelerating protocols while maintaining high yields of full-length cDNA. It is optimized for RNA input concentrations ranging from as low as 10 pg to as high as 5 µg of total RNA, enabling robust performance across various sample types. This kit is meticulously designed to enable rapid and efficient synthesis of high-quality cDNA, which can serve as a robust template for downstream applications, including real-time PCR, RNA quantification, and gene expression analysis, ensuring reproducible and reliable results with minimal effort. Its robust formulation provides exceptional stability and consistency, setting a new benchmark in cDNA synthesis. By combining the advanced performance of Supreme NZY Reverse Transcriptase with carefully selected premium components, Supreme NZY First Strand cDNA Synthesis Kit ensures a streamlined, reliable, and high-quality workflow for all your cDNA synthesis needs.

Shipping & Storage Conditions

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

**Shipped at room temperature for EU.*

This product contains proprietary reverse transcriptase enzymes, which are protected under intellectual property laws. Patent pending submitted by NZYtech.

Components

COMPONENT	MB50001 (50 reactions)		MB50002 (250 reactions)	
	TUBES	VOLUME	TUBES	VOLUME
Supreme RTase Enzyme Mix (1)	1	50 µL	5	50 µL
Supreme RTase Master Mix 5x (2)	1	200 µL	5	200 µL
RNase H (*E. Coli*)	1	250 µL	5	250 µL
DEPC-treated H_2O	1	1 mL	2	1 mL

(1) Includes Supreme NZY Reverse Transcriptase and Supreme NZY Ribonuclease Inhibitor
(2) Includes oligo(dT)$_{18}$, random hexamers, Mg^{2+} and dNTPs

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: All components are formulated to be ready to use.

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
Supreme RTase Master Mix 5x	4 µL
Supreme RTase Enzyme Mix	1 µL
total RNA; or mRNA / poly(A) RNA	10 pg – 5 µg 10 pg – 0.5 µg
DEPC-treated H_2O	up to 20 µL

Note: *In some cases, it might increase efficiency if the RNA is added before Master Mix and Enzyme Mix addition.*

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 60 °C for 1 min (this incubation can be adjusted from 30-70 °C for 1-10 min).
Notes
- *In Supreme NZY RT Enzyme Mix, present in the kit, 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 RNase H and incubate at 37 °C for 20 min.

Note: *Addition of 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.

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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

Supreme NZY Reverse Transcriptase and Supreme NZY Ribonuclease Inhibitor present in the Supreme RTase Enzyme Mix are >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Nucleases assay

All components of the kit are tested for nucleases 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 Supreme NZY First-Strand cDNA synthesis Kit 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.

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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.
• **Inadequate temperature of reverse transcription**
Reverse transcription can be conducted at 30-70°C. However, depending on the RNA template, we recommend temperature adjustment: - For complex RNA templates, with significant secondary structures, increase the temperature up to 70°C. - For longer RNA templates (> 0.5 kb) the optimal temperature is 50- 55°C.
• **Short incubation time for long targets**
For longer or complex RNA templates (> 0.5 kb), extend the reverse transcription incubation time from the standard 1 minute to 5-20 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
• **Non-specific Amplification**
Optimize reverse transcription conditions and implement hot start PCR strategies to minimize nonspecific amplification.
• **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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AMPLIFICATION IN THE NTC
• **Genomic DNA contamination**
Include a no-RT control to check for genomic DNA contamination. Apply DNase I treatment to RNA samples before reverse transcription to eliminate residual genomic DNA.

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For life science research only. Not for use in diagnostic procedures.

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