MB496_IFU_EN_V2402

Supreme NZY Reverse Transcriptase 200 U/µL

Catalogue number	Presentation
MB49601	100 µL (100 rxns of 20 µL)
MB49602	5 x 100 µL (500 rxns of 20 µL)

Features

- **High-Temperature Performance**: Optimized for temperatures between 42°C and 65°C, with a tolerance up to 70°C for improved reaction specificity.
- **Rapid cDNA Synthesis**: Completes cDNA synthesis in just 1 minute at 60°C, ideal for high-throughput applications.
- **High Stability, Accuracy and Processivity**: Reliably generates accurate full-length cDNA up to 9 kb, even in challenging RNA samples.

Description

Supreme NZY Reverse Transcriptase 200 U/µL is an advanced, second-generation reverse transcriptase (RT) developed for exceptional performance in a wide range of molecular biology applications. Designed for Research Use Only (RUO), this enzyme is optimized for high-speed and efficient cDNA synthesis at different temperatures (42°C to 70°C), making it an invaluable tool for researchers seeking reliable and reproducible results in their experiments. Supreme NZY Reverse Transcriptase 200 U/µL operates optimally at elevated temperatures ranging from 42°C to 65°C and can tolerate temperatures up to 70°C. For routine reverse transcription, 60°C is generally the optimal temperature, allowing for improved reaction specificity by reducing the formation of secondary structures in RNA templates and minimizing nonspecific priming, resulting in higher-quality cDNA synthesis. The enzyme is capable of synthesizing cDNA at an accelerated rate, completing reactions within 1 minute at 60°C, making it ideal for high-throughput workflows and time-sensitive applications. The enzyme lacks 3´→5´ exonuclease activity and has no RNase H activity, enabling the improved synthesis of full-length cDNA, even for long mRNA, using oligo dT priming. Supreme NZY Reverse Transcriptase 200 U/µL is highly processive, generating full-length cDNA molecules of up to 9 kb. Additionally, this enzyme exhibits remarkable stability, ensuring consistent performance over time and under various experimental conditions. Its robustness is particularly beneficial in challenging RNA samples, where enzyme stability is critical for successful reverse transcription. This enzyme is supplied in a glycerol-containing format, ensuring stability and ease of use in various reverse transcription protocols. For applications that require a glycerol-free format, NZYtech provides Supreme NZY Reverse Transcriptase 1 kU/µL (Cat. No. MB494). Supreme NZY Reverse Transcriptase is suitable for a broad spectrum of applications, including but not limited to first-strand cDNA synthesis, RT-PCR, RT-qPCR, and RNA-Seq library preparation. Its innovative design and enhanced features make it a preferred choice for researchers who require speed, specificity, and stability in their reverse transcription workflows. Supreme NZY Reverse Transcriptase 200 U/µL is produced through rigorous quality control (QC) processes, all conducted under the stringent guidelines of ISO 13485:2016, to safeguard its consistent performance and reliability.

Shipping & Storage Conditions

This product is shipped in dry ice to blue ice. Upon receipt, store all components at -85 °C to -15 °C in a constant temperature freezer. The product is designed to withstand a minimum of 10 freeze-thaw cycles without significantly losing performance. These meticulous storage procedures ensure that the Supreme NZY Reverse Transcriptase 200 U/µL delivers consistent and reliable results across its lifespan and usage. The kit will remain stable till the expiry date if stored as specified.

Components

COMPONENT		MB49601 (100 rxns)		MB49602 (500 rxns)	
		TUBES	VOLUME	TUBES	VOLUME
Supreme RTase 200 U/µL	Supreme NZY Reverse Transcriptase 200 U/µL	1	100 µL	5	100 µL
RxnBuffer 10x for Supreme RTase	Reaction Buffer 10x for Supreme NZY Reverse Transcriptase	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)$_{12-18}$ as a template-primer.

Enzyme concentration: 200 U/µL.

Inhibition & Inactivation: Supreme NZY Reverse Transcriptase 200 U/µL is inhibited in the presence of metal chelators (e.g. EDTA), inorganic phosphate, pyrophosphate and polyamines. The enzyme is inactivated at 85 °C for 5 min.

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 (A260/A280). Pure RNA should have an A260/A280 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. The provided reaction buffer is optimized with the appropriate Mg^{2+} concentration for Supreme NZY Reverse Transcriptase 200 U/µL activity. In rare cases, if reaction optimization requires it, additional Mg^{2+} can be added. If a precipitate is observed upon thawing the buffer, briefly vortex it until the precipitate is fully dissolved. To protect RNA from degradation and enhance cDNA yield, always include a Ribonuclease Inhibitor in the reaction, particularly when using ≤80 ng of RNA template. For Ribonuclease Inhibitors, refer to the NZYtech portfolio.

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 (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.
(**) To ensure optimal performance with gene-specific primers, we recommend adjusting the cDNA incubation temperature to the primer's melting temperature.

2. Mix and briefly centrifuge the reaction mixture.
3. Incubate at 65 °C for 5 min. After, chill the mixture on ice for at least 1 min. Briefly centrifuge and place on ice.
4. On ice, add the following components to the previous reaction mixture:

COMPONENT	1 REACTION VOLUME
RxnBuffer 10x for Supreme RTase	2 µL
NZY Ribonuclease Inhibitor (*)	1 µL (40 units)
Supreme RTase 200 U/µL	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.

5. Mix gently and centrifuge briefly.

6. Incubate at 60 °C for 1 min (this incubation can be adjusted from 42-70 °C for 1-5 min). For longer targets (> 0.5 kb) incubate for 10-20 min at 50-55 °C.
   Notes
   - *When using random-hexamer primers, incubate first at 25 °C for 10 min and then proceed with the 60 °C incubation (step 6).*
   - *The Supreme NZY Reverse Transcriptase 200 U/µL is fast enough to allow cDNA synthesis in as little as 1 minute. However, in certain cases (e.g., for longer or complex templates), increasing the incubation time up to 10 minutes may enhance cDNA yields.*

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

8. Immediately proceed to downstream applications or store cDNA at - 85 °C to -15 °C.
   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 200 U/µL 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 is incubated with 200 U of Supreme NZY Reverse Transcriptase 200 U/µL for 14-16h 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 the reaction buffer.

Functional assay

Supreme NZY Reverse Transcriptase 200 U/µL and respective buffer are tested for performance in an RT-qPCR experiment using a 10-fold serial dilution of total RNA from mouse liver (1 µg to 10 pg). The resultant cDNA is then used as a 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 and ensure the use of NZY Ribonuclease Inhibitor, especially with RNA inputs less than 50 ng. 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 (A260/A280 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 42-70°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.

When using gene-specific primers, optimizing the cDNA incubation temperature to the primer's melting point is highly recommended.

- **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.

- **Inadequate amount of Reverse Transcriptase**

Start with 200 U of Supreme NZY Reverse Transcriptase 200 U/µL for standard reactions, but adjust enzyme volume as needed for optimal results, particularly with long RNA templates.

- **Incorrect priming**

For long or complex templates, use a mix of random hexamers and oligo(dT) primers to improve cDNA yields.

- **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 ELECTROPHORESIS OR MULTIPLE PEAKS IN THE MELTING CURVE

- **Non-specific Amplification**

Optimize reverse transcription and priming conditions; consider using gene-specific primers 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.

AMPLIFICATION IN THE NTC

- **Genomic DNA contamination**

Include a no-RT control to check for genomic DNA contamination. Use primers that span exon-exon junctions and apply DNase I treatment to RNA samples before reverse transcription to eliminate residual genomic DNA.