MB501_IFU_EN_V2503

Supreme NZY One-Step RT-PCR Master Mix 2x

Catalogue number	Presentation
MB50101	2 x 1.25 mL (100 rxns of 50 µL)
MB50102	20 x 1.25 mL (1000 rxns of 50 µL)

Features

- **High Stability, Accuracy and Processivity:** Configures a reliable alternative for end-point PCR amplification of RNA templates even in challenging biological samples.
- **Superior Performance:** Designed to deliver high yields of PCR products up to 9 kb, even with low RNA levels, supporting downstream applications with unmatched accuracy and sensitivity.
- **Streamlined Workflow:** Provided as a one-tube 2x concentrated master mix, accelerating cDNA synthesis and amplification workflow while reducing potential errors.
- **Versatile Applications:** The amplified cDNA can be used for cloning, sequencing and gene expression studies.

Description

Supreme NZY One-Step RT-PCR Master Mix 2x is a robust all-in-one solution designed to deliver exceptional efficiency and sensitivity in cDNA synthesis and PCR amplification in end-point protocols. This ready-to-use formulation enables reverse transcription and PCR to be carried out sequentially in a single tube using gene-specific primers, streamlining experimental workflows and minimizing pipetting errors. At the core of the mix is Supreme NZY Reverse Transcriptase, a second-generation enzyme renowned for its exceptional stability, speed, accuracy, and reproducibility, ensuring high-quality, reliable RT-PCR results. The formulation also includes Supreme NZY Ribonuclease Inhibitor, which safeguards RNA integrity by protecting against RNase-mediated degradation throughout the reaction.

For effective cDNA amplification, the mix combines a specially engineered Taq DNA polymerase, optimized for high yield and reaction speed, with a proofreading enzyme that enhances specificity and supports the amplification of long cDNA templates with high accuracy. This unique enzyme blend enables amplification of fragments up to 9 kb, setting a new standard for performance in one-step RT-PCR. The proprietary buffer system supports a universal annealing temperature of 60 °C, significantly reducing the need for optimization across different targets and primer sets.

For downstream applications requiring high sensitivity, purification of the amplified product using NZYGelpure (MB011) or Speedy NZY ExoSAP Mix (MB489) is recommended. The amplified products are a mixture of predominantly A-overhang and some blunt-ended fragments, making them fully compatible with NZY-A PCR Cloning kits (MB053 or MB137).

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store the Master Mix 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 One-Step RT-PCR Master Mix 2x delivers consistent and reliable results across its lifespan and usage. The mix will remain stable till the expiry date if stored as specified.

Components

COMPONENT	SKU	TUBES	VOLUME
Supreme NZY One-Step RT-PCR Master Mix 2x	**MB50101 (100 rxns of 50 µL)**	2	1.25 mL
	MB50102 (1000 rxns of 50 µL)	20	1.25 mL

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: 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 (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: The Master Mix is formulated to be ready to use.
- Handling instructions: To maintain enzyme stability and prevent RNA degradation, keep all reagents on ice while setting up reactions. To reduce the risk of degradation, minimize the time that RNA is exposed, even on ice.
- Controls: For verification of the absence of contamination, prepare a mixture sample without RNA template (negative control). Additionally, include a positive control to serve as a reference for ensuring the correct functioning of the PCR reaction. If possible, use synthetic cDNA or a known cDNA sample to verify that reverse transcription and cDNA amplification are functioning correctly.

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 5 to 10% greater than the total required for the number of reactions to be performed.*
Note: *It is highly recommended to include a negative control reaction without RNA.*

COMPONENT	1 REACTION VOLUME / AMOUNT
Supreme NZY One-Step RT-PCR Master Mix 2x	25 µL
10 µM Forward Primer	1.75 µL
10 µM Reverse Primer	1.75 µL
Template RNA	1 µg – 0.001 pg total RNA
Molecular grade water	up to 50 µL

2. Mix and briefly centrifuge the reaction mixture.
3. Place the reaction plate/strip/tube within the thermal cycler and run the protocol defined below.
4. Analyse the PCR products through agarose gel electrophoresis (0.7-1.2%, w/v) and visualise with GreenSafe Premium (MB132) or any other means.

Suggested thermal cycling conditions

Supreme NZY One-step RT-PCR Master Mix 2x was optimized for the amplification of RNA fragments up to 9kb. The table below displays a standard RT-PCR setup optimized for this Master Mix.

CYCLES	TEMPERATURE	TIME	STAGE
1	55 °C	20 min	Reverse Transcription
1	94 °C	2 min	Initial Denaturation
30	94 °C	10 sec	Denaturation
	60 °C (*)	10 sec	Annealing
	68 °C	30 sec/kb (**)	Extension
1	68°C	5 min	Final Extension
	12°C	∞	Hold

(*) A universal annealing temperature of 60°C can be used for most primer pairs. However, for some specific primer pairs, an optimization of this temperature may be necessary.

(**) 30 sec/kb of extension time can be used for fragments up to 5kb. If exceeding this size, increasing the extension time to 1 min/kb is recommended.

Technical Notes

Primers: Primers should be designed to amplify specific cDNA regions, avoiding sequences with high homology to other genes to minimize non-specific amplification. Regions prone to secondary RNA structures should be avoided. It is also recommended that primers target exon-exon junctions to prevent genomic DNA amplification and ensure cDNA specificity. Primers should be 18-24 nucleotides long, with an ideal GC content of 40-60% for stable primer-template binding. Additionally, ensure that the forward and reverse primers do not form complementary sequences to prevent primer-dimer formation, which can reduce amplification efficiency.

$MgCl_2$: It is not necessary to supplement the reaction mixture with Mg^{2+} as the Supreme NZY One-step RT-PCR Master Mix 2x already contains an optimized concentration of $MgCl_2$.

Quality control

Genomic DNA contamination

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

Nucleases assay

Supreme NZY One-Step RT-PCR Master Mix 2x is tested for nuclease contamination, using 0.2-0.3 µg of pNZY28 plasmid DNA and 1 µg of RNA. 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

Supreme NZY One-Step RT-PCR Master Mix 2x is tested for performance in a RT- PCR for the amplification of different-sized DNA fragments. The resulting PCR products are visualized as single bands in a GreenSafe Premium-stained agarose gel.

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
• **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 / PCR 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 either a silica-based or magnetic beads-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 RT-PCR reaction. Reassess RNA quality and concentration using reliable quantification methods.
• **Inadequate temperature of reverse transcription**
Reverse transcription can be conducted at 55 °C. However, for complex RNA templates, with significant secondary structures, increasing the temperature up to 60 °C may be beneficial.
• **Short incubation time for long targets**
For longer or complex RNA templates (> 5 kb), prolong the extension time from the standard 30 sec/kb to 1 min/kb to enhance RT-PCR yield.
• **Primer degradation or insufficient concentration**
Store primers appropriately to prevent degradation. We recommend preparing small volume working aliquots from the stock solution. Avoid multiple freeze-thaw cycles. Confirm that primer design adheres to best practices. Optimize primer concentrations for efficient RT-PCR.
• **Inadequate annealing temperature or cycling conditions for cDNA amplification**
Ensure that PCR temperature profiles and cycling conditions are optimal for the assay. Validate and, if necessary, optimize the annealing and extension temperatures.
UNEXPECTED BANDS AFTER ELECTROPHORETIC ANALYSIS OF AMPLIFIED PRODUCTS
• **Non-specific Amplification**
Adjust annealing conditions and/or design another set of primers, by increasing the length and avoiding complementary sequences.
• **Contaminated Reagents or Equipment**
Use sterile, filtered tips, fresh reagents, and clean equipment regularly. Clean work surfaces with RNase/DNase decontaminants. Ensure that only molecular-grade water is used.
AMPLIFICATION IN THE NTC
• **Genomic DNA contamination**
Include a no-RNA control to check for genomic DNA contamination. Use primers that span exon-exon junctions or apply DNase I treatment to RNA samples before RT-PCR reaction to eliminate residual genomic DNA.