MB478_IFU_EN_V2402

NZYSupreme One-step RT-qPCR Probe Master Mix (2x), ROX

Catalogue number	Presentation
MB47801	2 x 1 mL (200 rxns of 20 µL)
MB47802	5 x 1 mL (500 rxns of 20 µL)
MB47803	20 x 1 mL (2000 rxns of 20 µL)

Description

NZYSupreme One-step RT-qPCR Probe Master Mix (2x), ROX, is an optimized and highly efficient reaction mixture developed for first-strand cDNA synthesis and subsequent real-time PCR, all in one single tube. This offers great convenience and minimizes the risk of errors and contaminations. The master mix also supports various types of assays, from single to triplex, providing experiment flexibility. Formulated as a 2× reaction mixture, it contains all components necessary for both cDNA synthesis and real-time PCR (including enzymes, dNTPs, stabilizers and enhancers), except primers, probes and RNA template. This master mix was engineered with a dual hot-start enzyme control mechanism to provide the highest detection sensitivity. In addition, the latest developments in PCR enhancers have been incorporated in the NZYSupreme One-step RT-qPCR Probe Master Mix, ROX, including buffer chemistry and incorporation of highly robust engineered enzymes and proteins. The inclusion of ROX reference dye in the master mix formulation does not interfere with the fluorescence emission of the reporter dyes commonly used in real-time PCR and provides advantages in the experiment. Despite most real-time PCR instruments that can read ROX dye, allowing users to run experiments and analyse data without ROX, the inclusion of this internal passive reference dye prevents data misinterpretation since it allows to normalize non-PCR-related fluctuations in fluorescence.

Shipping & Storage Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice. This master mix should be stored at -85 °C to -15 °C in a constant temperature freezer to guarantee maximal shelf life. Minimize the number of freeze-thaw cycles by storing it in working aliquots. Do not expose the master mix to direct sunlight. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	Cat. No.	TUBES	VOLUME
NZYSupreme One-step RT-qPCR Probe Master Mix (2x), ROX	**MB47801**	1	1 mL
	MB47802	5	1 mL
	MB47803	20	1 mL

Specifications

Compatibility with real-time PCR instruments

The master mix is compatible with instruments that measure the passive reference signal. Moreover, it is also compatible with instruments that do not require a passive reference signal for data normalization. The NZYSupreme One-step RT-qPCR Probe Master Mix (2x), ROX was optimized to be compatible with the following real-time PCR instruments:

Applied Biosystems: 7500; 7500 FAST; QuantStudio™ 6, 5, 7, 12k Flex & ViiA7™.

Sensitivity

NZYSupreme One-step RT-qPCR Probe Master Mix (2x), ROX, ensures high sensitivity in nucleic acid detection. In a 3-plex assay designed to detect two SARS-CoV-2 and one human endogenous RNA, the master mix demonstrates strong PCR efficiency within the range of 95-105%. Stringent testing across three master mix batches, using oropharyngeal samples, reveals an impressive limit of detection (LoD) at 0.25 copies/µL of SARS-CoV-2 viral RNA with a confidence level higher than 95%. This exceptional performance establishes it as a top choice for detecting low copy numbers, setting a new standard for reliable results in single to triplex reactions.

Multiplex capacity

Supporting nucleic acid analysis, NZYSupreme One-step RT-qPCR Probe Master Mix (2x), ROX, covers a spectrum from single to triplex assays, providing flexibility for diverse experimental requirements.

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: The inherent chemical instability of RNA and the ubiquitous presence of RNases require that particular care should be taken while working with RNA. We recommend using RNase-free plasticware/reagents and working in an RNase-free area (RNase Cleaner, Cat. No. MB16001, can help remove RNases from surfaces and materials).
- Handling instructions: To help prevent any carry-over DNA contamination, you should assign independent areas for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product should not be opened in the PCR set-up area. Use sterile filtered tips. Minimize exposure by keeping reaction and components capped whenever possible.
- Controls: For verification of the absence of contamination, prepare a mixture sample without a DNA template (negative control). To avoid incorrect results caused by unwanted fluorescent substances, consider adding a No-Amplification Control tube. This tube should have the sample but not the enzyme master mix. Elevated fluorescence in the No-Amplification Control compared to the No-Template Control suggests potential fluorescent contaminants in either the sample or the thermal cycler's heat block.

Procedure

The following protocol serves as a general guideline and a starting point for any One-step RT-qPCR procedure. Optimal reaction conditions (*e.g*. incubation times, temperatures and template concentration) may vary and, in particular conditions, may require further optimization.

1. Thaw the master mix at room temperature or on ice. Mix the master mix thoroughly by flicking the tube and inverting.
   Note: *A precipitate in the bottom of the master mix tube may be observed, in particular after multiple freeze/thaw cycles. To ensure optimal performance, please make sure all mix components are thawed and resuspended/homogenized before use. In this case, do not spin down the master mix before pipetting.*
2. In a clean reaction setup area, prepare the RT-qPCR reaction mixture according to the table below (please notice that the given volumes are based on a standard 20 µL final reaction mix and can be scale adjusted):
   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. Include sufficient reactions for the No-Templateand positive controls*
   Note: We recommend performing replicates of all reactions.

	1 REACTION VOLUME	FINAL CONCENTRATION
NZYSupreme One-step RT-qPCR Probe Master Mix (2x), ROX (1)	10 µL	1×
10 µM forward primer	0.8 µL	400 nM (2)
10 µM reverse primer	0.8 µL	400 nM (2)
10 µM probe	0.4 µL	200 nM (2)
Template	up to 8 µL (3)	-
Nuclease-free water	up to 20 µL	-

(1) If a slight precipitate is observed at the bottom of the master mix tube, please refer to the note above (step 1).
(2) Refer to the section of "Technical Notes" below for more details about primers and probes final concentrations in the reaction.
(3) To avoid cross-contamination, we strongly recommend pipetting the Template at last, preferably in a work separate area. For the No-Template Control, add Nuclease-free Water, instead of the template. For No-Amplification Control add Nuclease-free Water intead of master mix

3. Gently mix and centrifuge briefly to spin down the contents.
4. Pipette 12 µL of the Mix into each well, according to your experimental plate/strip/tube configuration.
5. – Pipette samples and controls: add up to 8 µL of RNA Sample in each well and add up to 8 µL of *Nuclease-free Water for negative control.*
6. Cover and seal the plate/strip/tube with appropriate caps or optical adhesive film before proceeding with the real-time PCR detection steps.
7. Centrifuge briefly to spin down the contents and eliminate any air bubbles from the reaction mixtures.
8. Place the reaction plate/strip/tube within the real-time PCR instrument and run the general protocol defined below. These conditions might be adapted to suit your specific needs, within sensible limits.

Suggested thermal cycling conditions

NZYSupreme One-step RT-qPCR Probe Master Mix, ROX, was optimized for the amplification of RNA fragments up to 200 bp under different RT-qPCR cycling conditions. The table below displays a standard 1-step RT-qPCR setup optimized on several platforms. However, these conditions may be adapted to suit different equipment-specific protocols.

CYCLES	TEMP.	TIME	CYCLE STEP
1	50-55 °C	10 - 20 min (*)	Reverse Transcription
1	95 °C	2-5 min	Polymerase activation
40	95 °C 60 °C	5 sec 30 - 60 sec	Denaturation Annealing/Extension

(*) Reverse transcriptase has a high processivity and may take as little as 10 minutes to synthesize cDNA. However, in specific situations, increasing reaction time up to 20 minutes may be beneficial.

Testing and Ct values

When comparing this RT-qPCR master mix with a mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of speed.

Technical Notes

Primers and probe: These guidelines refer to the design and set-up of dual labelled probes. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time RT-PCR can be critically affected by the sequence and concentration of the probes and primers, as well as by the amplicon length. We strongly recommend taking the following points into consideration when designing and running your real-time RT-qPCR experiment:

- Primers should have a melting temperature (Tm) of approximately 58-62 °C. The probe Tm should be approximately 10°C higher than that of the primers;
- The fragment to amplify should be between 80-200 bp in length and not superior to 300 bp;
- Final primer concentrations of 400 nM are suitable for most probe-based reactions. However, to determine the optimal concentration we recommend titrating in the range 150-600 nM. Forward and reverse primers concentration should be equimolar;
- A final probe concentration of 200 nM is suitable for most applications; we recommend that the final probe concentration is at least two-fold lower than the primer concentration; to determine the optimal concentration we recommend titrating in the range 50-300 nM;
- For multiplex RT-qPCR, high probe concentrations can result in cross-channel fluorescence. For high-complexity multiplex assays, we highly recommend utilizing the NZYSupreme Multiplex One-Step RT-qPCR Probe Master Mix. (Cat. No. MB442).

Template: The RNA template must be purified and devoid of contamination by RT-qPCR inhibitors (*e.g.* EDTA). The recommended amount of template is dependent upon the source of RNA used. Please consider the following points when selecting RNA templates:

- **Total RNA:** purified total RNA can be used in the range from 1 pg to 5 µg per 20 µL reaction.
- **mRNA:** purified mRNA can be used from 0.01 pg per 20 µL reaction.

To obtain a high yield of highly purified RNA we suggest using the NZY Total RNA Isolation Kit (Cat. No. MB134).

$MgCl_2$: It is not necessary to supplement the reaction mixture with $MgCl_2$ as the NZYSupreme One-step RT-qPCR Probe Master Mix, ROX already contains an optimized concentration of $MgCl_2$.

RT-qPCR optimization: It may be necessary to improve the efficiency of some reactions, such as multiplexing with more than two probes, or if the target amplicon is longer than 200 bp. In these cases, the reverse transcription reaction time can be extended up to 30 minutes; the annealing/extension time can be extended up to 60 seconds.

Quality control assays

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time PCR.

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the master mix for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the master mix 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.

Functional assay

NZYSupreme One-step RT-qPCR Probe Master Mix, ROX is extensively tested for activity, processivity, efficiency, sensitivity and heat activation.