NZYSupreme qPCR Probe Master Mix (2x), ROX plus

Catalogue number:

MB43901, 2 mL (200 x 20 µL)
MB43902, 5 mL (500 x 20 µL)
MB43903, 20 mL (2000 x 20 µL)

Description

NZYSupreme qPCR Probe Master Mix (2x), ROX plus is an optimized and highly efficient reaction mixture developed for real-time PCR, specifically for probe-detection technology (including molecular beacons). 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 qPCR Probe Master Mix, including buffer chemistry and incorporation of highly robust engineered enzymes. 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. The presence of ROX reference dye in the master mix enables to increase confidence in data analysis, since it allows to normalize non-PCR-related fluctuations in fluorescence. Despite the majority of real-time PCR instruments that are able to read ROX dye allow users to run experiments and analyse data without ROX, the inclusion of this internal passive reference dye prevents data misinterpretation and allows to detect and diagnose errors.
NZYSupreme qPCR Probe Master Mix (2x), ROX plus is provided as a simple-to-use, stabilized 2x reaction mixture that includes all components for quantitative PCR, except sample DNA, primers, probe and water.

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice.

Storage Conditions

This master mix should be stored at -85°C to -15°C in a freezer without defrost cycles in order to guarantee maximal shelf life. Minimize the number of freeze-thaw cycles by storing in working aliquots. The product will remain stable till the expiry date if stored as specified.

Compatible real-time PCR instruments

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

Applied Biosystems:
7000; 7300; 7700; 7900; 7900HT; 7900HT FAST; StepOne™ & StepOne™plus

Protocol

The following protocol serves as a general guideline and a starting point for any qPCR procedure. Optimal reaction conditions (incubation times and temperatures, concentration of template DNA) may vary and, in particular conditions, may require further optimization.

qPCR reaction set-up: the given volumes are based on a standard 20 µL final reaction mix which can be scale adjusted.

NZYSupreme qPCR Probe Master Mix (2x), ROX plus	10 µL	1×
10 µM forward primer	0.8 µL	400 nM(*)
10 µM reverse primer	0.8 µL	400 nM(*)
10 µM probe	0.2 µL	100 nM(*)
Template	up to 8 µL	-
Nuclease-free water	up to 20 µL	-

(*) *See section of "General considerations" below for more details about primers and probes final concentrations in the reaction.*

Testing and Ct values: When comparing NZYSupreme qPCR Probe Master Mix (2x), ROX plus 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.

Suggested thermal cycling conditions

NZYSupreme qPCR Probe Master Mix (2x), ROX plus was optimized for the amplification of DNA fragments up to 200 bp under different real-time PCR cycling conditions. The table below displays a standard set-up optimized on a number of platforms. However, these conditions may be adapted to suit different machine-specific protocols.

Cycles	Temp.	Time	Notes
1	*95 °C	*2-5 min	Polymerase activation
40	95 °C	5 sec	Denaturation
	60 °C	**30 sec – 50 sec	Annealing/Extension

** 2 min for cDNA, up to 3 or 5 min for genomic DNA.*

*** Up to 50 sec may be necessary for multiplexing with more than two probes.*

General considerations

To prevent any DNA contamination, we recommend that users have independent areas for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area.

Primers and probe: these guidelines refer to the design and set-up of TaqMan probe-based PCR. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time 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 PCR experiment:

- Primers should a melting temperature (T_m) of approximately 60°C. The probe T_m should be approximately 10 °C higher than that of the primers
- The fragment should be between 80-200 bp length and not superior to 300 bp
- Final primer concentration of 400 nM is suitable for most probe-based reactions. However, to determine the optimal concentration we recommend titrating in the range 0.2-1 µM. The forward and reverse primers concentration should be equimolar
- A final probe concentration of 100 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 0.1-0.25 µM;
- For multiplex qPCR, probe concentrations in excess of 100 nM can result in cross channel fluorescence.

Template: It is important that the DNA template is purified and concentrated according to conventional nucleic acid clean up procedures. In addition, templates must be devoid of any contaminating PCR inhibitors (e.g. EDTA). The recommended amount of template for PCR is dependent upon the type of DNA used. Please consider the following points when using genomic DNA or cDNA templates:

- **Genomic DNA:** use up to 1 µg of genomic DNA in a single PCR. We recommend using NZY Tissue gDNA Isolation kit (Cat. No. MB135) for high yield and purity from both prokaryotic and eukaryotic sources.
- **cDNA:** the optimal amount of cDNA to use in a single PCR depends upon the copy number of the target gene; we suggest using 100 ng cDNA per reaction. However, this amount may be adjusted to a more appropriate concentration. We suggest using NZY First-Strand cDNA Synthesis Kit (Cat. No. MB125) for reverse transcription of purified RNA. To obtain high yield of highly purified RNA we suggest using NZY Total RNA Isolation Kit (Cat. No. MB134).

$MgCl_2$: It is not necessary to supplement the mix with $MgCl_2$ as NZYSupreme qPCR Probe Master Mix (2x), ROX plus already contains an optimized concentration of $MgCl_2$.

PCR controls: The reliability of the data may be affected by the presence of contaminating DNA. It is strongly recommended to include a no-template control reaction in the qPCR design, replacing template DNA/cDNA with nuclease-free PCR-grade water (DEPC-treated Water, Cat. No. MB43701). When performing a two-step RT-PCR, set up a no-RT control as well as a no-template control for the PCR.

Quality control assays

Genomic DNA contamination

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

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 qPCR Probe Master Mix (2x), ROX plus is extensively tested for activity, processivity, efficiency, sensitivity and heat activation.

V2401

For research use only.