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

Catalogue number: MB22801, 2 mL (200 x 20 µL)
MB22802, 5 mL (500 x 20 µL)
MB22803, 20 mL (2000 x 20 µL)

Description

NZYSpeedy qPCR Probe Master Mix (2x), ROX plus is an optimized and highly efficient reaction mixture developed for real-time PCR. This master mix enables fast and highly reproducible procedures on the most common real-time PCR apparatus. This kit was developed for probe-detection technology, including molecular beacons. The latest developments in PCR enhancers have been incorporated in this master mix, including buffer chemistry and a polymerase with hot start like activity. These combinations guarantee that NZYSpeedy qPCR Probe Master Mix, ROX plus delivers sensitivity coupled with highly reproducible and fast real-time PCR protocols. NZYSpeedy qPCR Probe Master Mix (2x), ROX plus is provided as a 2× reaction mixture containing all components necessary for real-time PCR, including dNTPs, stabilizers and enhancers.

Storage temperature

NZYSpeedy qPCR Probe Master Mix (2x), ROX plus should be stored at -20 °C, in a constant temperature freezer. Minimize the number of freeze-thaw cycles by storing in working aliquots.

Compatible real-time PCR instruments

The master mix was developed to be 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.

NZYSpeedy qPCR Probe Master Mix (2x), ROX plus has been 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.

Reaction mix composition: the given volumes are based on a standard 20 µL final reaction mix which can be scale adjusted.

NZYSpeedy 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 8.2 µL	
Nuclease-free water	as required	

Testing and Ct values: When comparing NZYSpeedy 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

NZYSpeedy qPCR Probe Master Mix (2x), ROX plus was optimized for the amplification of DNA fragments up to 200 bp. The table below displays a standard setup 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	10 s	Denaturation
	60 °C	**20-50s	Annealing/Extension (acquiring at end of step)

*2 min for cDNA, up to 5 min for genomic DNA
**Up to 50s may be necessary for multiplexing with more than two probes.

General considerations

In order 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 have 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.

NOTE: For multiplex qPCR, the probe concentrations in excess of 100 nM can result in cross channel fluorescence.

Template: It is important that the DNA template is purified and may be concentrated according to conventional nucleic acid clean up procedures (NZYGelpure, MB011). In addition, the template 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 (MB135) for high yield and purity from both prokaryotic and eukaryotic sources.

- **cDNA:** the optimal amount of cDNA to use in a single PCR is dependent upon the copy number of the target gene; we suggest using 100 ng cDNA per reaction. However, it may be necessary to vary this amount performing a two-step RT-PCR. We suggest using NZY First-Strand cDNA Synthesis Kit (MB125) for reverse transcription of purified RNA. To obtain high yield of highly purified RNA we suggest using the NZY Total RNA Isolation Kit (MB134).

$MgCl_2$: It is not necessary to supplement the reaction mixture with $MgCl_2$ as NZYSpeedy 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, so it is important to detect it. We suggest that you always include a no-template control reaction, replacing the template with PCR-grade water. 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

NZY qPCR Master Mixes (2x) must be free of any detectable genomic DNA contamination as evaluated through real time PCR.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with NZY qPCR Master Mixes (2x) for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid.

Functional assay

NZY qPCR Master Mixes (2x) are extensively tested for activity, processivity, efficiency, sensitivity and heat activation.

Certificate of Analysis

Test	Result
gDNA contamination	Pass
Nuclease contamination	Pass
Functional assays	Pass

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