MB228_IFU_EN_V2501

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

Catalogue number	Presentation
MB22801	2 x 1 mL (200 rxns of 20 µL)
MB22802	5 x 1 mL (500 rxns of 20 µL)
MB22803	20 x 1 mL (2000 rxns of 20 µL)
MB22805	1 x 50 mL (5000 rxns of 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 into 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. The inclusion of the internal passive reference dye prevents data misinterpretation and allows to detect and diagnose errors. This product has been validated across all common real-time instruments that require a high concentration of the passive reference dye. It also supports various types of assays, from single to triplex, providing experiment flexibility.

Shipping & Storage Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice. Upon arrival, all components 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	SKU	TUBES/BOTTLES	VOLUME
NZYSpeedy qPCR Probe Master Mix (2x), ROX plus	**MB22801**	1	1 mL
	MB22802	5	1 mL
	MB22803	20	1 mL
	MB22805	1	50 mL

Note: Consider preparing multiple aliquots of the master mix to reduce freeze/thaw cycles and minimize the risk of contamination.

Specifications

Compatibility with real-time PCR instruments

NZYSpeedy qPCR Probe Master Mix (2x), ROX plus 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. It was optimized to be compatible with the following real-time PCR instruments:

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

Sensitivity

The master mix incorporates a hot-start mechanism to effectively suppress non-specific amplification, resulting in greater sensitivity and consistency in assay performance. It is particularly suited for precise quantification of targets present in low abundance, even in limited sample volumes.

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: Stringent precautionary measures must be imposed to mitigate the risk of carry-over contamination of DNA. We recommend using DNase-free plasticware/reagents and working in a DNase-free area (Nucleases & Nucleic Acid Cleaner, Cat. No. MB48301, or DNA & RNA Cleaner, Cat. No. MB46201, can help remove DNases from surfaces and materials).

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- 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. Any tubes containing amplified PCR product mustn't be opened in the PCR set-up area. Use sterile filtered tips. Minimize exposure by keeping reaction and components capped whenever possible.
- Controls: To verify the absence of contamination, prepare a mixture sample without a DNA template (negative control). Additionally, include a positive control to serve as a reference for ensuring the correct functioning of the qPCR reaction and detection system. The positive control should exhibit the expected amplification and/or fluorescence signal, confirming the assay's ability to accurately detect the target sequence. 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 is a general guideline and a starting point for any 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.
2. In a clean reaction setup area, prepare the 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 1: *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 negative and positive controls.*
   Note 2: *If necessary, prepare a No-Amplification Control by adding nuclease-free water instead of the qPCR master mix.*
   Note 3: We recommend performing replicates of all reactions.

	1 REACTION VOLUME	FINAL CONCENTRATION
NZYSpeedy qPCR Probe Master Mix (2x), ROX plus	10 µL	1×
10 µM forward primer	0.8 µL	400 nM (1)
10 µM reverse primer	0.8 µL	400 nM (1)
10 µM probe	0.4 µL	200 nM (1)
FINAL VOLUME =	up to 12 µL (2)	-

(1) Refer to the section of "Technical Notes" below for more details about primers and probes final concentrations in the reaction.
(2) If using smaller volumes for the primers & probe, supplement the volume up to 12 µL with Nuclease-free Water.

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 template (samples and controls):
   a. Add up to 8 µL of DNA sample/positive control into each respective well. If using less volume than 8 µL, supplement with Nuclease-free Water up to 8 µL. In total, the final volume of the reaction will be 20 µL.
   b. Add up to 8 µL of Nuclease-free Water for the negative control to achieve a final volume of 20 µL.

Note: *To avoid cross-contamination, we strongly recommend pipetting the template at last, preferably in a work separate area.*

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

NZYSpeedy 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 qPCR setup optimized on several platforms. However, these conditions may be adapted to suit different equipment-specific protocols.

CYCLES	TEMP.	TIME	STAGE
1	95 °C	2 - 5 min(*)	Polymerase activation
40	95 °C 60 °C	5 -10 sec 20 - 50 sec (**)	Denaturation Annealing/Extension

(*) 2 min for cDNA, up to 3 or 5 min for genomic DNA.
(**) Amplicons exceeding 200 base pairs in length, or multiplex assays with more than two probes, may require up to 50 sec.

Testing and Ct values

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

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Technical Notes

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 (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 70-200 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 150-200 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 of 50-300 nM;
- For multiplex RT-qPCR, high probe concentrations can result in cross-channel fluorescence.

Template: The DNA template must be purified and devoid of contamination by PCR inhibitors (*e.g.* EDTA). The recommended amount of template is dependent upon the source of DNA used. Please consider the following points when selecting genomic DNA or cDNA templates:

- **Genomic DNA:** use up to 1 µg of genomic DNA in a single PCR. We recommend using the 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 the NZY First-Strand cDNA Synthesis Kit (Cat. No. MB125) for reverse transcription of purified RNA. To obtain a 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 reaction mixture with $MgCl_2$ as the 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. 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.

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

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

This master mix is manufactured under stringent quality standards and complies with ISO 9001 and ISO 13485 certifications for research and diagnostic-grade reagents.

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