MB223_IFU_EN_V2501

NZYSpeedy qPCR Green Master Mix (2x), ROX

Catalogue number	Presentation
MB22301	2 x 1 mL (200 rxns of 20 µL)
MB22302	5 x 1 mL (500 rxns of 20 µL)
MB22303	20 x 1 mL (2000 rxns of 20 µL)
MB22305	1 x 50 mL (5000 rxns of 20 µL)

Description

NZYSpeedy qPCR Green Master Mix (2x), ROX 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. 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 Green Master Mix (2x), ROX delivers ultra-sensitivity coupled with highly reproducible and fast real-time PCR protocols. The master mix is provided as a 2x reaction mixture that contains all components necessary for real-time PCR, including a green intercalating dye, dNTPs, stabilisers and enhancers. 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 most real-time PCR instruments that can read ROX dye allow users to run experiments and analyze data without ROX, the inclusion of this internal passive reference dye prevents data misinterpretation and allows to detect and diagnose errors. NZYSpeedy qPCR Green Master Mix (2x), ROX is ready-to-use and only requires primers and template addition. It is optimized for intercalating green dye detection on different instruments.

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. The green dye is light sensitive, as such the master mix should be protected from light whenever possible. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	SKU	TUBES/BOTTLES	VOLUME
NZYSpeedy qPCR Green Master Mix (2x), ROX	**MB22301**	1	1 mL
	MB22302	5	1 mL
	MB22303	20	1 mL
	MB22305	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 Green Master Mix (2x), ROX 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™: 7500; 7500 FAST; QuantStudio™ 6, 7, 12k Flex & ViiA7™.

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).
- 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: For verification of 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.

- Replicates: It is highly recommended performing replicates of each reaction; we recommend performing four replicates or at least three.

Protocol

The following protocol serves as 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.
   Note: *To ensure optimal performance, please make sure all mix components are thawed and resuspended/homogenized before use. Before pipetting, mix vigorously the master mix by inverting the tube and then spin down.*
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.*
   Note4: *To avoid cross-contamination, we strongly recommend pipetting the template at last, preferably in a work separate area.*

	1 REACTION VOLUME	FINAL CONCENTRATION
NZYSpeedy qPCR Green Master Mix (2x), ROX	10 µL	1×
10 µM forward primer	0.8 µL	400 nM (1)
10 µM reverse primer	0.8 µL	400 nM (1)
Template	up to 8.4 µL	-
Nuclease-free water	as required	-
FINAL VOLUME =	20 µL	-

(2) Refer to the section of "Technical Notes" below for more details about primers final concentrations in the reaction.

3. Gently mix and centrifuge briefly to spin down the contents.
4. Cover and seal the plate/strip/tube with appropriate caps or optical adhesive film before proceeding with the real-time PCR detection steps.
5. Centrifuge briefly to spin down the contents and eliminate any air bubbles from the reaction mixtures.
6. 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 Green Master Mix (2x), ROX 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 setup optimized on several platforms. However, these conditions may be adapted to suit different machine-specific protocols.

CYCLES	TEMP.	TIME	STAGE
1	95 °C (*)	2 min (*)	Polymerase activation
40	95 °C 60-65 °C	5 sec 15-30 sec (**)	Denaturation Annealing/Extension (acquiring at end of step)

(*) 2 min for cDNA, up to 3 or 5 min for genomic DNA.
(**) Recommendation: combined annealing/extension should be lower than 30 seconds.

Melting curve analysis: At the end of the qPCR run, it is highly recommended to perform a melting curve. A melt curve performed after qPCR cycling with an intercalating dye will typically produce a single distinct peak. This indicates that the amplified double-stranded DNA products are a single discrete species. The presence of multiple DNA species in the same reaction produces multiple peaks in the melt curve, typically indicating the presence of contaminating or off-target amplification products. Follow the manufacturer's instructions for the real-time PCR instrument for melting curve analysis

Testing and Ct values

When comparing NZYSpeedy qPCR Green Master Mix (2x), ROX 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, increasing $MgCl_2$ concentration to 6 mM may reduce Cts.

Technical Notes

Primers: The specific amplification, yield and efficiency of any real-time PCR can be affected by both sequence and primers concentration, as well as by the fragment length. We strongly recommend taking the following suggestions into consideration when designing and running your real-time PCR experiment:

- Primers should have a melting temperature (T_m) of approximately 58-62 °C;
- The fragment length should be between 70-200 bp and not superior to 400 bp;
- Final primer concentration of 400 nM is suitable for most *green* reactions. However, to determine the optimal concentration we recommend titrating in the range 100-1000 nM. The forward and reverse primers concentration should be equimolar;
- Design intron spanning primers when amplifying from cDNA (to avoid gDNA amplification).

Template: The DNA template must be purified and devoid of contamination by PCR inhibitors (*e.g.* EDTA). It is important that the DNA template is purified and concentrated according to conventional nucleic acid clean-up procedures (NZYGelpure, MB011). 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).

$MgCl_2$: NZYSpeedy qPCR Green Master Mix (2x), ROX contains $MgCl_2$ at a concentration of 3 mM, in the final 1x reaction mix, which is an optimal concentration for most real-time PCR procedures.

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.

Green intercalating dye NZYSpeedy qPCR Green Master Mix (2x), ROX contains a non-specific double-strand DNA-binding dye, that will bind to all dsDNA fragments present in the reaction. Upon binding to DNA, it emits green fluorescence (λ = 520 nm) while showing no detectable inhibition to the PCR reaction.

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. 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 Green Master Mix (2x), ROX 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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