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

Catalogue number:

MB22201, 2 mL (200 x 20 µL)
MB22202, 5 mL (500 x 20 µL)
MB22203, 20 mL (2000 x 20 µL)

Description

NZYSpeedy qPCR Green 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. 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 plus 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. NZYSpeedy qPCR Green Master Mix (2x), ROX plus is ready-to-use and only requires primers and template addition. It is optimized for intercalating green dye detection on different instruments.

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. The green dye is light sensitive, as such the master mix should be protected from light whenever possible.

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 NZYSpeedy qPCR Green 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 may require further optimization in particular conditions.

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

NZYSpeedy qPCR Green 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
Template	up to 8.4 µL	
Nuclease-free water	as required	

Testing and Ct values: When comparing NZYSpeedy qPCR Green 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. For difficult amplicons, increasing $MgCl_2$ concentration to 6 mM may reduce C_ts.

Suggested thermal cycling conditions

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

Cycles	Temp.	Time	Notes
1	*95 °C	*2 min	Polymerase activation
40	95 °C 60-65 °C	5 s **15-30s	Denaturation Annealing/Extension (acquiring at end of step)

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

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: 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 60 °C.
- The fragment length should be between 80-200 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 0.1-1 µM. The forward and reverse primers concentration should be equimolar.

- Design intron spanning primers when amplifying from cDNA (to avoid gDNA amplification).

Template: It is important that the DNA template is purified and concentrated according to conventional nucleic acid clean up procedures (NZYGelpure, MB011). 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 (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).

$MgCl_2$: NZYSpeedy qPCR Green Master Mix (2x), ROX plus 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, 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. Furthermore, refer to the instrument instructions for the option of melt-profile analysis.

Quality control assays

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

Nuclease assay
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.

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For research use only.

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