NZYSupreme qPCR Green Master Mix (2x)

Catalogue number:

MB41901, 2 mL (200 x 20 µL)
MB41902, 5 mL (500 x 20 µL)
MB41903, 20 mL (2000 x 20 µL)

Description

NZYSupreme qPCR Green Master Mix (2x) is an optimized and highly efficient reaction mixture developed for real-time PCR. This master mix was engineered with a 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 Green Master Mix, including buffer chemistry and incorporation of highly robust engineered enzymes. These combinations guarantee that NZYSupreme qPCR Green Master Mix (2x) delivers ultra-sensitivity coupled with highly reproducible and fast real-time PCR protocols. It was designed to amplify targets for accurate gene expression analysis. 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, stabilizers and enhancers. NZYSupreme qPCR Green Master Mix (2x) is ready-to-use and only requires primers and DNA 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 does not contain any passive reference dye and thus it is compatible with real-time PCR instruments that do not require a passive reference signal for data normalization. NZYSupreme qPCR Green Master Mix (2x) was optimized to be compatible with the following real-time PCR instruments:

Bio-Rad®: CFX96™; CFX384™; Opticon™; Opticon™ 2

Qiagen (Corbett): Rotor-Gene™ 3000, 6000 & Q

Roche: Lightcycler® 96, 480 & Nano

Applied Biosystems (with optional ROX addition or ROX OFF):
7000; 7300; 7700; 7900; 7900HT; 7900HT FAST; StepOne™ & StepOne™plus; 7500; 7500 FAST; QuantStudio™ 5, 6, 7, 12k Flex & ViiA7™

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 Green Master Mix (2x) (*) (**)	10 µL	1×
10 µM forward primer	0.8 µL	400 nM (¥)
10 µM reverse primer	0.8 µL	400 nM (¥)
DNA Template	up to 8.4 µL	-
Nuclease-free water	as required	-

(*) Before pipetting, mix vigorously the master mix by inverting the tube and then spin down.

(**) The master mix does not contain ROX, but, if required, addition of this internal passive reference dye can be conducted in a separate step. The final concentration will vary according to the qPCR instrument used. Please follow instructions described in the section "ROX reference dye" below.

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

***Note:** Before distributing the reaction mixture into the qPCR tubes/plate, mix thoroughly by inversion and then centrifuge briefly.*

Replicates and Controls: It is highly recommended performing replicates of each reaction; we recommend performing four replicates or at least three. In addition, introduce negative controls – No Template Control (NTC) reactions contain all reaction components except template and are important to detect qPCR contamination (they should not originate a Ct value).

Testing and Ct values: When comparing NZYSupreme qPCR Green Master Mix (2x) 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.

ROX reference dye: NZYSupreme qPCR Green Master Mix (2x) is compatible with most thermocyclers available in the market and can include ROX passive reference dye (not provided) to normalize non-PCR-related fluctuations in fluorescence. If ROX addition is required for your qPCR platform, an optimal quantity of this dye should be included in your master mix. The recommended amount of ROX for the most common qPCR instruments is stated in the table below:

qPCR instrument	Volume of ROX per mL of Master Mix (2x) (*)
Applied Biosystems: 7000/7300/7700/7900/7900HT/7900HT FAST/ StepOne™/StepOne™plus	20-29 µL (500-725 nM)
Applied Biosystems: 7500/7500FAST/QuantStudio™ 5, 6, 7, 12k Flex/ViiA7™	2-4 µL (50-100 nM)
Bio-Rad®: CFX96™/CFX384™/iCycler®/iQ™5/Opticon™/ Opticon™ 2 **Qiagen:** Rotor-Gene™ 3000/6000/Q **Roche:** Lightcycler® 96/480/Nano	Not required

(*) For different volumes please scale-up or scale-down the volume of ROX accordingly. Please notice that ROX is not provided by NZYTech.

Suggested thermal cycling conditions

NZYSupreme qPCR Green Master Mix (2x) 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 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	15-30s	Annealing/Extension

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

Melting curve analysis: At the end of the qPCR run, it is highly recommended performing 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 manufacturer's instructions of the real-time PCR instrument for melting curve analysis.

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: 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:

- 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).
- **$MgCl_2$:** It is not necessary to supplement the mix with $MgCl_2$ as NZYSupreme qPCR Green Master Mix (2x) 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. Furthermore, refer to the instrument instructions for the option of melt-profile analysis.

Green intercalating dye: NZYSupreme qPCR Green Master Mix (2x) 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 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 Green Master Mixes are extensively tested for activity, processivity, efficiency, sensitivity and heat activation.

V2202

Certificate of Analysis

Test	Result
Genomic DNA contamination	Pass
Nuclease assays	Pass
Functional assay	Pass

Approved by: 

Patrícia Ponte

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