NeoSupreme qPCR Green Master Mix (2x), ROX

#Cat: NB-60-0007 Size: 1mL

Description

NeoSupreme qPCR Green Master Mix (2x), ROX 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 NeoSupreme me qPCR Green Master Mix, ROX, including buffer chemistry and incorporation of highly robust engineered enzymes. These combinations guarantee that NeoSupreme qPCR Green Master Mix (2x), ROX delivers ultrasensitivity 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. 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 analyse data without ROX, the inclusion of this internal passive reference dye prevents data misinterpretation and allows to detect and diagnose errors. NeoSupreme qPCR Green Master Mix (2x), ROX 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 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 NeoSupreme qPCR Green Master Mix (2x), ROX was optimized to be compatible with the following real-time PCR instruments:
Applied Biosystems: 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

NeoSupreme qPCR Green Master Mix (2x), ROX (*)	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	-

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

Suggested thermal cycling conditions

NeoSupreme 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 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 60 °C	5 sec 15-30 sec	Denaturation 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 (Tm) 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 (NeoGelPure, NB-60-0002). 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.
- **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. To obtain high yield of highly purified RNA
- **$MgCl_2$:** It is not necessary to supplement the mix with $MgCl_2$ as NeoSupreme qPCR Green Master Mix (2x), ROX 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: NeoSupreme 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 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

NeoSupreme qPCR Green Master Mixes are extensively tested for activity, processivity, efficiency, sensitivity and heat activation.

Certificate of Analysis	
Test	**Result**
Genomic DNA contamination	**Pass**
Nuclease assays	**Pass**
Functional assay	**Pass**
Approved by:  **Patrícia Ponte** **Senior Manager, Quality Systems**	

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