

qNTQX® qPCR SYBR Green Master Mix (No Rox)

Cat. no. LM601003

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Product Information

Cat. No.	Product name	Pack size
LM601003	qNTQX® qPCR SYBR Green Master Mix (No Rox)	5 × 1 mL
		100 × 1 mL

Please state the required pack size when ordering.

Kit Contents

Contents	5 × 1 mL	100 × 1 mL
2× qPCR SYBR Green Master Mix (No Rox)	5 × 1 mL	100 × 1 mL

Storage

Store protected from light at –25 °C to –15 °C. Shelf life is 18 months. Avoid repeated freeze-thaw cycles. This product contains the fluorescent dye SYBR Green I; protect from strong light during storage and reaction set-up.

Introduction

qNTQX® qPCR SYBR Green Master Mix (No Rox) is a 2× premix for real-time quantitative PCR, containing hot-start Hieff® DNA Polymerase, SYBR Green I dye, dNTPs and Mg²⁺. Only template and primers need to be added to the reaction, greatly simplifying set-up and reducing the risk of contamination.

The DNA polymerase is coupled to a ligand that regulates polymerase activity in real time as a function of temperature, keeping the enzyme inactive at room temperature and active during thermal cycling. The formulation additionally includes factors that suppress non-specific amplification and factors that improve amplification efficiency, giving reliable linearity across a wide quantification range.

Features

1. Ready-to-use 2× SYBR Green master mix — only primers, template and water need to be added.
2. Ligand-regulated hot-start DNA polymerase for high specificity and low background.
3. Optimised formulation suppresses non-specific amplification and extends the linear quantification range.
4. No ROX reference dye — validated on Qiagen Rotor-Gene and other non-ROX-normalised qPCR platforms (see Protocol, section E).
5. Compatible with two-step and three-step cycling protocols, both concluding with a melt-curve stage for specificity confirmation.

Notes Please be sure to read this precaution before using the kit.

1. This product is for scientific research use only. Not for use in diagnostic or clinical procedures.
2. For RT-qPCR from RNA, we recommend our cDNA synthesis kit (Cat. no. 11141) to effectively remove residual genomic DNA from the RNA sample.

- Flocculent material may appear in the Master Mix after thawing; this does not affect performance. Keep at 4 °C and mix by inversion until the solution is clear.
- Avoid repeated freeze-thaw cycles of the Master Mix.
- For your safety and health, please wear a laboratory coat and disposable gloves while handling this product.

Protocol

A. Reaction Set-up

Component	Volume (µL)	Volume (µL)	Final concentration
2× qPCR SYBR Green Master Mix (No Rox)	25	10	1×
Forward primer (10 µM)	1	0.4	0.2 µM
Reverse primer (10 µM)	1	0.4	0.2 µM
Template DNA	X	X	—
RNase-free H ₂ O	to 50	to 20	—

Mix thoroughly before use, avoiding vigorous shaking and excessive bubble formation. Primer concentration is typically 0.2 µM final, adjustable between 0.1–1.0 µM. A 20 µL or 50 µL total reaction volume is recommended for reliable, reproducible target amplification. If the template is undiluted cDNA stock, its volume should not exceed 1/10 of the total reaction volume; a 5–10× dilution is recommended, aiming for a Ct value of 20–30 cycles.

B. Recommended Cycling Programmes

1. Two-step protocol (higher specificity)

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	5 min	1
Denaturation	95 °C	10 s	40
Annealing / extension	60 °C	30 s	
Melt curve	Instrument default	—	1

2. Three-step protocol (higher amplification efficiency)

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	5 min	1
Denaturation	95 °C	10 s	40
Annealing	55–60 °C	20 s	
Extension	72 °C	20 s	
Melt curve	Instrument default	—	1

Two-step cycling is preferred for higher specificity; three-step cycling is preferred for higher amplification efficiency. Initial denaturation can be shortened to 2 min depending on template and primers. Annealing temperature/time should be adjusted for primer and target length. Fluorescence acquisition time should follow the instrument manufacturer's instructions; typical settings are ≥30 s (Applied Biosystems StepOne/StepOnePlus/7500 Fast, Roche LightCycler 480, Bio-Rad CFX96), ≥31 s (Applied Biosystems 7300) or ≥34 s (Applied Biosystems 7500). The melt-curve stage can generally use the instrument's default settings.

C. Data Analysis

Amplification curve / Ct value: at least three biological replicates are recommended per quantitative experiment; confirm both the amplification curve and the melt curve before interpreting results.

1. Ct 20–30: most accurate quantification range.
2. Ct < 10: dilute the template and repeat the reaction.
3. Ct 30–35: increase template concentration or reaction volume to improve amplification efficiency and result reliability.
4. Ct > 35: results cannot be reliably quantified, though they may still support qualitative (presence/absence) interpretation.

Melt curve:

1. A single melt peak indicates good reaction specificity, suitable for quantitative analysis; two or more peaks indicate the result is not suitable for quantitative analysis.
2. If a second peak is present, run agarose gel electrophoresis to determine whether it represents primer dimer or non-specific amplification.
3. If primer dimer: reduce primer concentration, or redesign primers with better amplification efficiency.
4. If non-specific amplification: increase the annealing temperature, or redesign primers with higher specificity.

D. Primer Design Guidelines

1. Recommended primer length is approximately 25 bp; recommended amplicon length is 100–300 bp, ideally around 150 bp.
2. The T_m of the forward and reverse primers should not differ by more than 2 °C; a T_m of 60–65 °C is preferred.
3. Bases should be evenly distributed along the primer, avoiding runs of 4 or more identical bases, with GC content around 50%; the last 3' base is ideally G or C.
4. Avoid 3 or more complementary bases within a single primer or between the forward and reverse primers.
5. Check primer specificity with NCBI BLAST, and avoid 2 or more non-specific complementary bases at the primer 3' end.
6. Verify amplification efficiency for each primer pair — only primer pairs with matched amplification efficiencies should be used for quantitative comparison between targets.

E. Compatible Instruments

Bio-Rad: CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4.

Eppendorf: Mastercycler ep realplex, realplex 2s.

Qiagen: Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000.

Roche Applied Science: LightCycler 480, LightCycler 2.0, LightCycler 96.

Thermo Scientific: PikoReal Cyclers.

Others: Cepheid SmartCycler; Illumina Eco qPCR.

This No-Rox formulation is intended for qPCR platforms that do not require ROX passive reference dye normalisation. For instruments that require ROX normalisation (e.g. certain Applied Biosystems platforms), please ask us about the Low Rox Plus / High Rox Plus variants of this Master Mix.