qNTQX® qPCR SYBR Green Master Mix (Antibody, No Rox)

Cat. no. LM601006

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Product Specification

Product	qNTQX® qPCR SYBR Green Master Mix (Antibody, No Rox)
Catalog No.	LM601006
Storage conditions	Store at −25 °C to −15 °C, protected from light. The product has a shelf life of 18 months. Avoid repeated freeze–thaw cycles. Since the product contains the fluorescent dye SYBR Green I, avoid exposure to strong light when storing or preparing the reaction system.
Size	1 mL / 5 × 1 mL / 5 × 5 mL / 10 × 5 mL / 20 × 5 mL
Application	**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 Cycler **Cepheid:** SmartCycler **Illumina:** Eco qPCR

Product Description

This product is a 2× premixed solution for real-time quantitative PCR amplification. It uses antibody-based hot-start DNA polymerase, where heating at the pre-denaturation temperature (95 °C) for 30 seconds inactivates the Taq antibody, releasing the active Taq DNA polymerase. The antibody-based hot-start Taq enzyme effectively suppresses non-specific primer annealing, preventing unwanted amplification. Additionally, the formulation has been optimised with factors that effectively inhibit non-specific PCR amplification and enhance the amplification efficiency, making it suitable for the amplification of low-concentration templates. This ensures that quantitative PCR can produce a good standard curve over a wide quantitative range.

The mix contains Taq DNA polymerase, Taq antibody, SYBR Green I, dNTPs, and Mg^{2+}. For use, simply add the template and primers to the amplification system for real-time fluorescent quantitative PCR, greatly simplifying the operation and reducing the risk of contamination.

Protocol

A. Reaction System (Recommended to Prepare on Ice)

Component	Volume (μL)	Volume (μL)	Final concentration
qNTQX® qPCR SYBR Green Master Mix (Antibody, No Rox)[a)]	25	10	1×
Forward primer (10 μM)[b)]	1	0.4	0.2 μM
Reverse primer (10 μM)[b)]	1	0.4	0.2 μM
Template DNA[c)]	X	X	—
RNase-free H_2O	to 50	to 20	—

a) Before use, ensure thorough mixing of the master mix, avoiding vigorous shaking to prevent the formation of excessive bubbles.
b) Primer concentration: the final primer concentration is typically 0.2 μM, but it can be adjusted between 0.1–1.0 μM based on the

experiment.
c) Template DNA: if the template is undiluted cDNA, the volume should not exceed 1/10 of the total qPCR reaction volume. It is recommended to dilute cDNA 5–10 times. The ideal template amount is one that results in a Ct value between 20–30 cycles.
d) Recommended reaction volumes: it is suggested to use a total volume of 20 µL or 50 µL to ensure the effectiveness and reproducibility of the target gene amplification.

B. Amplification Procedure[a)]

1. Two-step method

Cycle step	Temperature	Time	Number of cycles
Pre-denaturation[b)]	95 °C	30 s	1
Denaturation	95 °C	10 s	40
Annealing/extension[c)]	60 °C	30 s[d)]	
Melting curve stage[e)]	Instrument default settings	—	1

2. Three-step method

Cycle step	Temperature	Time	Number of cycles
Pre-denaturation[b)]	95 °C	30 s	1
Denaturation	95 °C	10 s	40
Annealing[c)]	55–60 °C	20 s	
Extension	72 °C	20 s[d)]	
Melting curve stage[e)]	Instrument default settings	—	1

a) The two-step method is ideal for high specificity, while the three-step method is preferable for high-efficiency amplification.
b) Pre-denaturation: time can be increased to 3–5 minutes depending on the template and primers.
c) Annealing temperature and time: should be adjusted based on primer and target gene length.
d) For fluorescence signal collection, follow the instrument's instructions. Common settings: ≥30 s (Applied Biosystems StepOne, StepOnePlus, 7500 Fast; Roche LightCycler 480; Bio-Rad CFX96); ≥31 s (Applied Biosystems 7300); ≥34 s (Applied Biosystems 7500).
e) Melting curve can typically be performed using the instrument's default program.

C. Results Analysis

At least three biological replicates are required for quantitative experiments. After the reaction, confirm the amplification and melting curves.

Amplification curve

1. A Ct value between 20–30 ensures accurate quantitative analysis.
2. If the Ct value is less than 10, dilute the template and repeat the experiment.
3. If the Ct value is between 30–35, increase template concentration or reaction volume to improve amplification efficiency and ensure accuracy.
4. If the Ct value is above 35, the result cannot be used for quantitative gene expression analysis but can be used for qualitative analysis.

Melting curve

1. A single peak indicates good reaction specificity, allowing for quantitative analysis. Multiple peaks indicate the reaction is not suitable for quantitative analysis.
2. If a double peak appears, perform agarose gel electrophoresis to determine if the non-target peak is a primer dimer or non-specific amplification.

3. If it is a primer dimer, reduce primer concentration or redesign primers with higher amplification efficiency.
4. If it is non-specific amplification, increase the annealing temperature or redesign primers with higher specificity.

D. Primer Design Guidelines

1. The recommended primer length is around 25 bp, with an ideal amplification product length of 150 bp, and can range from 100 bp to 300 bp.
2. The Tm values of the forward and reverse primers should not differ by more than 2 °C. A Tm of 60–65 °C is optimal.
3. Primers should have evenly distributed bases, avoiding sequences with 4 consecutive identical bases. GC content should be around 50%, and the 3′ end should preferably end with G or C.
4. Avoid complementary sequences of 3 or more bases within a primer or between the forward and reverse primers.
5. Use NCBI BLAST to check primer specificity and avoid more than 2 base pairs of non-specific complementarity at the 3′ end.
6. Test the amplification efficiency of designed primers. Only primers with the same amplification efficiency should be used for comparative quantitative analysis.

Notes

1. After thawing, the Master Mix may appear flocculent; place at 4 °C and mix by inverting until the solution is clear. This does not affect reagent performance.
2. It is recommended to use the company's cDNA synthesis kit to effectively remove genomic DNA residues from RNA samples.
3. This product is for research use only.
4. For your safety and health, wear a lab coat and disposable gloves during operation.