

qNTQX® Advanced qPCR SYBR Master Mix

Cat. no. LM601001

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Product Information

Cat. No.	Product name	Size
LM601001	qNTQX® Advanced qPCR SYBR Master Mix	1 mL
		5 × 1 mL
		50 × 1 mL
		100 × 1 mL

Storage and Shipping

1. The product is shipped with an ice pack.
2. The product can be stored at -15°C to -25°C for 18 months.
3. The product contains fluorescent dyes; avoid strong light irradiation when storing or preparing the reaction system.

Applicable Instruments

Applied Biosystems: 7500, 7500 Fast, ViiA7, QuantStudio 1, 3 and 5, QuantStudio 6, 7, 12K Flex.

Stratagene: MX3000P, MX3005P, MX4000P.

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

qNTQX® Advanced qPCR SYBR Master Mix is a 2× ready-to-use premix for real-time quantitative PCR amplification. It offers high fluorescence intensity, high sensitivity and specificity, and high amplification yield. It is blue in colour and includes a loading tracer to facilitate sample addition. The core component, qNTQX® Taq DNA polymerase, uses an antibody-based hot-start mechanism that effectively suppresses non-specific amplification caused by primer annealing during sample preparation. In addition, the formulation includes a factor that improves PCR amplification efficiency and a promoter factor that balances the amplification of genes with different GC contents (30–70%), so that quantitative PCR can achieve a good linear relationship across a wide quantitative range.

Protocol

A. qPCR Reaction System

Components	Volume (μL)	Volume (μL)	Final Conc.
qNTQX® Advanced qPCR SYBR Master Mix	10	25	1×

Components	Volume (μL)	Volume (μL)	Final Conc.
Forward Primer (10 μmol/L) ^{a)}	0.4	1	0.2 μmol/L
Reverse Primer (10 μmol/L) ^{b)}	0.4	1	0.2 μmol/L
DNA/cDNA template ^{b)}	—	—	—
ddH ₂ O	up to 20	up to 50	—

a) Primer concentration: the final primer concentration is 0.2 μmol/L, and can also be adjusted between 0.1 and 1.0 μmol/L as appropriate.

b) If the template is undiluted cDNA stock solution, the volume used should not exceed 1/10 of the total volume of the qPCR reaction. The optimal amount of template added should ensure that the Ct value obtained by amplification falls within 20–30 cycles.

c) It is recommended to use 20 μL or 50 μL to ensure the effectiveness and reproducibility of target gene amplification; mix thoroughly before use on the instrument to avoid excessive bubbles caused by vigorous shaking.

B. Reaction Program

Cycle step	Temp.	Time	Cycles
Initial denaturation	95 °C	2 min	1
Denaturation	95 °C	10 sec	35
Annealing/Extension ^{a)}	60 °C	30 sec ^{b)}	
Melting curve stage ^{c)}	Instrument defaults	—	1

a) Annealing temperature and time: please adjust according to the length of the primer and target gene.

b) Fluorescence signal acquisition: please set the run according to the requirements in the instrument's instructions for use. The time setting for several common instruments is as follows: 20 sec — Applied Biosystems 7700, 7900HT, 7500 Fast; 31 sec — Applied Biosystems 7300; 32 sec — Applied Biosystems 7500.

c) Melting curve: the instrument default program can normally be used.

Primer Design Guide

1. The recommended primer length is about 25 bp. The optimal length of the amplified product is 150 bp, and can be selected from 100 bp to 300 bp.
2. The difference in T_m values of the forward and reverse primer should not exceed 2 °C. The optimal primer T_m value is 60 °C – 65 °C.
3. Primer bases should be distributed evenly to avoid 4 consecutive identical bases, and the GC content should be controlled around 50%. The last base at the 3' end is preferably G or C.
4. It is best to avoid complementary sequences of more than 3 bases within a primer or between the forward and reverse primers.
5. Primer specificity should be checked using the NCBI BLAST program, to avoid non-specific complementarity of more than 2 bases at the 3' end of the primer.
6. Designed primers need to be tested for amplification efficiency. Only primers with matching amplification efficiency can be used for quantitative comparative analysis.

Notes

1. For your safety and health, please wear a lab coat and disposable gloves during operation.
2. After thawing, the Master Mix may appear flocculent or show a white precipitate. Dissolve it slowly by hand and mix gently by inverting up and down until the solution is clear. This does not affect the performance of the reagent.
3. It is recommended to use our company's cDNA synthesis kit (Cat. No.: LM502002) to effectively remove residual genomic DNA from RNA samples.

4. If electrophoresis is required, to obtain clear bands, it is recommended to dilute the qPCR product 20–30 fold before electrophoresis.
5. This product is for research use ONLY.