

qNTQX® Advanced One Step RT-qPCR SYBR Green Kit

Cat. no. LM601011

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1. Product Specification

Item	Details
Product	qNTQX® Advanced One Step RT-qPCR SYBR Green Kit
Catalog No.	LM601011
Size	20 T / 200 T
Storage conditions	This product should be stored at –25 °C to –15 °C for 1 year.

2. Product Description

qNTQX® Advanced One Step RT-qPCR SYBR Green Kit is a kit for fluorescence quantification based on SYBR Green I dye. Using gene-specific primers, reverse transcription and qPCR reactions are completed in one tube without repeated open-cap and pipetting steps, greatly increasing detection efficiency and reducing the risk of contamination. For RNA samples, the kit uses heat-resistant qNTQX® Reverse Transcriptase to efficiently synthesise cDNA, together with quantitative amplification by qNTQX® HotStart Taq DNA Polymerase. In the optimised buffer system, the detection sensitivity of the kit against a highly expressed target can reach 0.1 pg, and a moderately expressed target can reach 1 pg. The kit is also suitable for amplification and quantification of DNA samples, and can achieve highly sensitive detection and quantification of nucleic acids from different animal and plant samples, cells and microorganisms.

3. Instructions

3.1. Recommended Reaction System

Components	Volume (µL)	Volume (µL)	Final concentration
2× qNTQX® Advanced SG Buffer	12.5	25	1×
qNTQX® Advanced UH Enzyme Mix	—	2	—
Forward primer (10 µM)	0.5	1	0.2 µM
Reverse primer (10 µM)	0.5	1	0.2 µM
Template RNA	X	X	—
RNase-free ddH ₂ O	Up to 25	Up to 50	—

3.2. Reaction Programme

Cycle step	Temp.	Time	Cycles
Reverse transcription	50 °C	6 min	1
Predenaturation	95 °C	5 min	1
Denaturation	95 °C	15 sec	40
Amplified reaction	60 °C	30 sec	
Melt-curve phase	Instrument default settings	—	1

3.3. Applicable Instruments

Instrument models without ROX correction:

Bio-Rad: CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4; Eppendorf: Mastercycler ep realplex, realplex2s; 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; Cepheid: SmartCycler; Illumina: Eco qPCR.

Low ROX applicable models:

ABI 7500, 7500 Fast, ViiA 7, QuantStudio 3 and 5, QuantStudio 6, 7, 12K Flex; Stratagene MX3000P, MX3005P, MX4000P.

High ROX applicable models:

ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne, StepOne Plus.

3.4. Primer Design Techniques

1. Primers should ideally be designed to span an exon-exon junction, where one of the amplification primers potentially crosses the actual exon-intron boundary; this design reduces the risk of false-positive amplification from contaminating genomic DNA.
2. Primers should be specific. After primer design, a BLAST search should be performed.
3. Primer length is generally 18–27 bp, and should not be too long, otherwise the extension temperature will be too high and unsuitable for the Taq DNA polymerase reaction.
4. The GC content of primers should range from 40% to 60%; either too high or too low a GC content is unfavourable for the reaction, and the GC content of the forward and reverse primers should not differ too much. The T_m value of the primers (the temperature at which 50% of the oligonucleotide duplex is unwound at a given salt concentration) should generally be 5–10 °C above the effective annealing temperature. The T_m value can be estimated using $T_m = 4(G + C) + 2(A + T)$, or calculated using dedicated software.
5. PCR product length is usually controlled to 80–300 bp; primer design should take amplification efficiency into account.
6. Avoid an A at the 3' end of the primer where possible; a 3'-end mismatch has a large effect on efficiency (A causes little reduction even when mismatched, T causes a large reduction, and G/C mismatches fall in between), so T is generally preferred at the 3' end.
7. Bases should be randomly distributed without runs of purines or pyrimidines; in particular, the 3' end should not contain more than three consecutive G or C bases, as this raises the error rate in GC-rich sequence regions.
8. Avoid complementary sequences within a single primer or between the two primers, which could otherwise fold into a hairpin structure or form primer dimers through steric hindrance at the 3' overlapping ends; no more than 3 consecutive complementary bases should exist between primers, and if a hairpin or primer-dimer structure is unavoidable its ΔG should not exceed 4.5 kcal/mol, otherwise primer-dimer bands will form, reducing the effective primer concentration and disrupting the PCR reaction.

3.5. Abnormal Outcome Analysis

1) No Ct value appears

1. Error in fluorescence signal collection: collect the signal at the end of the annealing/extension step.
2. Primer degradation: check primer integrity by PAGE electrophoresis.
3. Insufficient template amount: samples of unknown concentration should be tested from the stock solution.

4. Template degradation: avoid introducing impurities and avoid repeated freeze-thaw cycles during sample preparation.

2) Large Ct value (Ct > 35)

1. Low amplification efficiency: the reaction conditions are not sufficiently optimised or the primer design has issues; a three-step programme can be tried, and the annealing temperature can be reduced appropriately.
2. PCR product too long: a product length of 80–150 bp is generally recommended.

3) Poor linearity of the standard curve

1. Sample addition error: use non-gradient standards where possible; for RNA templates, gradient dilution with 1× TE buffer is recommended, and for DNA templates, dilution with ddH₂O is recommended.
2. Standard degradation: avoid repeated freeze-thaw cycles of the standards, or re-prepare and dilute the standards.
3. Poor primer design: redesign the primers.
4. Inhibitors present in the template, or the template concentration is too high.

4) The NTC (no-template control) is amplified (Ct < 32)

1. The primer design is not sufficiently optimised: avoid the formation of primer dimers and hairpin structures.
2. Primer concentration is not well matched: reduce the primer concentration appropriately, and check the concentration ratio of the forward and reverse primers.
3. Aerosol contamination: leakage of amplification products easily causes aerosol contamination in the laboratory, which leads to inaccurate quantitative results once it occurs. Use of a DNA/RNA remover for nucleic-acid aerosol decontamination is recommended.

4. Notes

1. Sample addition and dispensing steps should be performed on ice as far as possible.
2. Each component should be mixed by shaking and briefly centrifuged before use.
3. For your safety and health, please wear a laboratory coat and disposable gloves during operation.
4. This product is for research use only.