



SMOBIO

Small Bio, Smart Tool

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Product Information

ExcelTaq™ series

2X Q-PCR Master Mix (SYBR, no ROX)

TQ1100 200 RXN

2X Q-PCR Master Mix (SYBR, no ROX) 1 ml x 2

TQ1101 500 RXN

2X Q-PCR Master Mix (SYBR, no ROX) 1 ml x 5

Storage

Aliquot to avoid multiple freeze-thaw cycles

Protect from light

-20°C for 12 months

Features

- High sensitivity and signal intensity
- More compatible with reverse transcriptase
- With smart blue contrast dye as a visual aid for reaction setup
- Low background

Description

The ExcelTaq™ 2X Q-PCR Master Mix (SYBR, no ROX) is a ready-to-use reagent with all the essential components for quantitative real-time PCR (qPCR) except primers and templates. The master mix features high sensitivity (Fig. 1) and signal intensity as well as low background and better compatibility with cDNA templates derived directly from reverse transcriptase reaction mixture (Fig. 2).

The ExcelTaq™ 2X Q-PCR Master Mix (SYBR, no ROX) contains hot-start *Taq* polymerase in an optimized buffer with dsDNA specific SYBR green fluorescent dye. This master mix allows sensitive, precise amplification, real-time tracking of the amplification process, and simultaneous quantification for targeted DNA molecules.

With inert smart blue contrast dye, the ExcelTaq™ 2X Q-PCR Master Mix (SYBR, no ROX) is ready-to-use and greatly reduces pipetting errors, while largely improving the reproducibility of the process. The ExcelTaq™ 2X Q-PCR Master Mix (SYBR, no ROX) is also compatible with ROX reference dye if ROX is recommended by the manufacturer of the qPCR system.

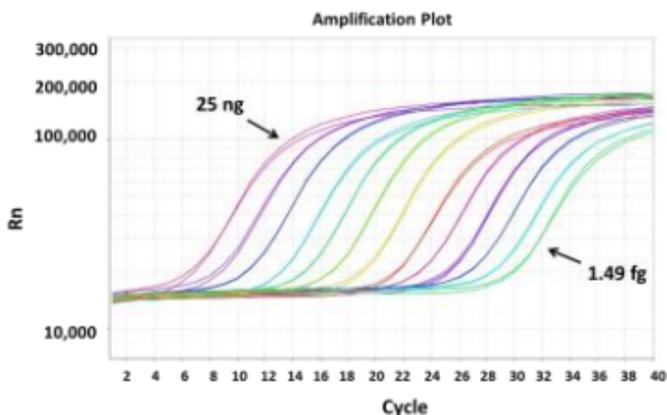


Fig. 1. The amplification plot of real-time PCR with cDNA template ranged from 25 ng to 1.49 fg in quantity, analyzed by using TQ1100 ExcelTaq™ 2X Q-PCR Master Mix (SYBR, no ROX) for qPCR amplification.

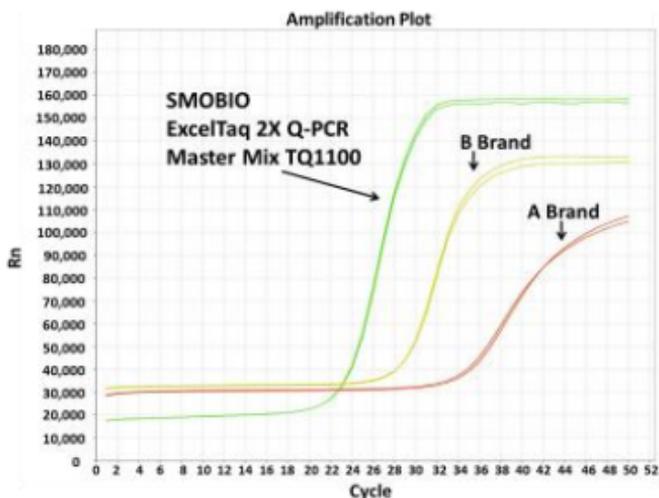


Fig. 2. SMOBIO's TQ1100 ExcelTaq™ 2X Q-PCR Master Mix (SYBR, no ROX) shows better compatibility with reverse transcriptase as compared with similar products from A and B brands. Two μ l of cDNA directly obtained from reverse-transcription reaction mixture were used in a 20 μ l qPCR reaction for the compatibility test.

Application

- Quantitative real-time PCR
- Quantitative two-step real-time PCR

Instrument compatibility

- BioRad system:
 - CFX96
 - Chromo 4™ Real-Time Detector
 - DNA Engine Opticon™
 - DNA Engine Opticon™ 2
 - CFX384 Touch
- Cepheid system:
 - Smart Cycler®
- Eppendorf system:
 - Mastercycler® ep realplex
- Roche system:
 - Roche LightCycler® 480
 - Roche LightCycler® Nano
- QIAGEN system:
 - Rotor-Gene™ Q
- Illumina system:
 - Eco™

Note: **ExcelTaq™ 2X Q-PCR Master Mix (SYBR, no ROX)** is compatible with a variety of real-time instruments, including but not limited to the list above.

Recommended primer design

- Amplicon size: 80-150 bp
- T_m value: around 60°C (calculated with Primer3 software)
- Primer length: 17-25 mer
- Sequence:
 - 45-55% of GC content is recommended.
 - Avoid regional high GC or AT content
 - Avoid palindrome sequence
 - Sequence with G or C at the 3' end is recommended.
- Specificity of primers should be confirmed through a BLAST search.

Recommended reaction mixture set up for qPCR

| | |
|------------------------------------|---------------|
| Template | 2 µl* |
| Forward primer | 50 – 400 nM** |
| Reverse primer | 50 – 400 nM** |
| 2X Q-PCR Master Mix (SYBR, no ROX) | 10 µl |
| H ₂ O | to 20 µl |
| <hr/> | |
| Total volume | 20 µl |

*Final template concentration varies depending on the copy number of target present in the template solution. The recommended amount of template is: **100 fg -100 ng of cDNA, 80 pg -50 ng of gDNA or 10²-10⁸ molecules of plasmid.**

**The PCR primer concentration for an optimal qPCR reaction may vary according to primers' properties and template condition.

Recommended qPCR program

Try Two-step cycle protocol first, and optimize the reaction conditions if necessary. If the two-step protocol still does not give optimal results (e.g., if T_m values for the primers are low), try the Three-step cycle protocol.

Two-step cycle for qPCR

| Steps | Temp. | Time | Cycles |
|---|----------------------------|---------------------|--------|
| Template denature and enzyme activation | 95°C | 10 min [#] | 1 |
| Denature | 95°C | 15 sec | 40 |
| Annealing/Extension | 60°C | 60 sec | |
| Melting curve analysis | Refer to instrument manual | | |

Three-step cycle for qPCR

| Steps | Temp. | Time | Cycles |
|---|----------------------------|---------------------|--------|
| Template denature and enzyme activation | 95°C | 10 min [#] | 1 |
| Denature | 95°C | 15 sec | 40 |
| Annealing | 55-60°C | 30 sec | |
| Extension | 72°C | 30 sec | |
| Melting curve analysis | Refer to instrument manual | | |

[#] We suggest 10 minutes for the first step to thoroughly denature DNA and activate enzymes.

Other Information

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Caution: Not intended for human or animal diagnostic or therapeutic uses.

Related Products

| | |
|--------|--|
| RP1000 | ExcelRT Reverse Transcriptase, 20,000 U |
| RP1100 | ExcelRT One-step RT-PCR Kit, 50 RXN |
| RP1400 | ExcelRT Reverse Transcription Kit II, 100 RXN |
| RI1000 | RNAok RNase Inhibitor, 2000 U |
| TQ1110 | ExcelTaq 2X Q-PCR Master Mix (SYBR, ROX), 200 RXN |
| TQ2110 | ExcelTaq 2X Q-PCR Master Mix (TaqMan, ROX), 200 RXN |
| DM2300 | ExcelBand 100 bp+3K DNA Ladder, 500 μ l |
| DM3100 | ExcelBand 1 KB (0.25-10 kb) DNA Ladder, 500 μ l |
| DL5000 | FluoroDye DNA Fluorescent Loading Dye (Green, 6 \times), 1 ml |
| NS1000 | FluoroVue Nucleic Acid Gel Stain (10,000X), 500 μ l |
| PM2510 | ExcelBand Enhanced 3-color Regular Range Protein Marker, 250 μ l \times 2 |
| TF1000 | SMO-HiFi DNA Polymerase, 100 U \times 1 |
| TP1000 | ExcelTaq Taq DNA Polymerase, 500 U \times 1 |
| TP1200 | ExcelTaq 5X PCR Master Dye Mix, 200 RXN |
| CK1000 | Champion E. coli Transformation Kit |
| WM1000 | YesBlot Western Marker I, 250 μ l |