



**Certificate
of Analysis**

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Chai Inc.
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Product Name: **Chai Green qPCR Master Mix**
Catalog Num: R02201
Lot Num: 0157205
Analysis Date: Jul 25, 2019
Expiration Date: Jul 25, 2021
Storage: Store at -20 °C

Master Mix Tests

Results

PCR Amplification Test

Efficiency and R² were determined using a five-point standard curve with ten-fold serial dilutions of bacteriophage lambda genomic DNA as template. An amplicon of 200 bp was amplified using cycling conditions of 2 min @ 95 °C, 40x (15 s @ 95 °C, 60 s @ 60 °C). The efficiency is specified to be between 90–110% and R² ≥ 0.99.

PASS

Endonuclease Activity Test

Absence of endonuclease activity was determined by overnight incubation of *E. coli* amplified DNA with 25 U Taq polymerase in 1X reaction buffer at 37 °C, and monitored for little to no decrease in original amount of amplicon when resolved by gel electrophoresis.

PASS

Hot Start Functional Test

Hot Start function of Taq polymerase was tested using bacteriophage lambda genomic DNA template and a gene fragment containing the mouse GAPDH gene. Cycling conditions of 2 min @ 95 °C, 40x (15 s @ 95 °C, 60 s @ 60 °C) were used. The products were resolved by agarose gel electrophoresis on a 4% gel. Decreased primer dimer formation and absence of non-specific amplification using Chai Green qPCR Master Mix compared to a variant not containing hot start function was monitored.

PASS

ROX Reference Dye Tests

Results

ROX Emission Spectrum Test

Fluorescence emission spectrum of ROX reference dye was measured at 1 nm intervals, at a scan speed of 6000 nm/min using 580 nm excitation wavelength. The peak emission wavelength is specified to be 600 ± 5 nm.

PASS

ROX Performance Test

Efficiency and R^2 were determined using a five-point standard curve with ten-fold serial dilutions of bacteriophage lambda genomic DNA as template and ROX as passive reference dye. An amplicon of 200 bp was amplified using cycling conditions of 2 min @ 95 °C, 40x (15 s @ 95 °C, 60 s @ 60 °C). The efficiency is specified to be between 90 – 110% and $R^2 \geq 0.99$.

PASS