

RT-qPCR was performed using a Bio-Rad CFX96 Real-Time system (C1000 Touch Thermal Cycle, Bio-Rad, Singapore). Reactions were performed in a 10 μ L reaction mixture containing 5 μ L PowerUp SYBR Green Master-Mix 2X (A25779, Applied Biosystems), 2 μ L of 10 ng cDNA, 1 μ L of each specific primer (10 μ M), and 1 μ L of molecular-grade water. The PCR conditions were as follows: 50°C for 2 min; 40 cycles of 95°C for 2 min, 95°C for 15 s, 57°C for 15 s, 72°C for 1 min; and an extra step of melting analysis for each sample at 65°C for 5 s and 95°C for 5 s. Gene amplification was performed in duplicates, and each PCR run was performed thrice. A negative control (ultrapure water) was used in each assay.