Real-time polymerase chain reaction (qPCR)

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Abstract

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Protocol

Step 1.

Total RNA was extracted from cell lines Trizol (Invitrogen Life Technologies), according to the manufacturer’s instructions.

Step 2.

Next, the first strand cDNA was synthesized from RNA by reverse transcription using PrimeScript™ RT reagent Kit (TaKaRa Bio Group, Dalian, China), according to the manufacturer’s instructions.

Step 3.

Real-time PCR was performed using the CXF96 (Bio-Rad, CA, USA) system and a SyberGreen II real-time PCR kit (TaKaRa). The PCR reaction conditions were as follows: denaturation at 95°C for 3 min, followed by 39 cycles at 95°C (30 s), 59°C (30 s) and 72°C (30 s), and a final extension at 72°C for 5 min.

Step 4.

All statistical analyses were performed using Microsoft Office Excel.