Real-time RT-PCR

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Abstract

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Protocol

Total RNA isolation

Step 1.

Total RNA was isolated from MSCs with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA).

Reverse transcription

Step 2.

Reverse transcription reactions contained 2 µg RNA, random hexamers or oligo (dT), and reverse transcriptase, and were performed according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA).

Real-time PCR

Step 3.

Real-time PCR was performed with QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany) and an Icycler iQ Multi-color Real-time PCR Detection System. The reactions were run in duplicate with 1 µl of cDNA template in a 20 µl reaction volume with the program running at 95°C for 15 min, followed by 40 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s. The amplification specificity was confirmed by melting curve. The mRNA level of the target gene was acquired from the value of threshold cycle (Ct) as a relative level to that of GAPDH through the formula 2 -△Ct (△Ct = GAPDH Ct - gene of interest Ct). The efficiency of the primers was confirmed by sequencing the conventional PCR products before applying for real-time PCR.