Real-time PCR

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Abstract

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Protocol

Total RNA isolation

Step 1.

The bilateral TGs from four rats per group were dissected and pooled for total RNA isolation using TRIzol reagent (Invitrogen, Carlsbad, CA, USA).

Reverse transcription

Step 2.

Reverse transcription PCR was conducted with an iScript cDNA synthesis kit (Bio-Rad) in 20 μl reaction volume containing 1 μ[1]g of total RNA incubated at 25°C for 5 min, transcripted at 42°C for 30 min, and terminated by heating at 85°C for 5 min. The synthesized cDNA was stored at -20°C until use.

Real-time PCR

Step 3.

Real-time PCR was performed with Power SYBR Green PCR Master Mix (Applied Biosystems) using a 7500 real-time PCR System (Applied Biosystems). The reactions were run in duplicate with 1 μl of cDNA template in a 20 μl reaction volume with the program running at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 94°C for 15 s and 60°C for 1 min. 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 β–actin through the formula 2 -△Ct (△Ct = β-actin Ct - gene of interest Ct). The efficiency of the primers was confirmed by sequencing the conventional PCR products before applying for real-time PCR.