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Real-time PCR

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Total RNA isolation

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

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

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.