qPCR of α-synuclein, TNF and NF-κβ

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ABSTRACT

A procedure for quantitative real time reverse transcription PCR of α-synuclein, TNF and *NF*-κβ

MATERIALS

β-*actin*
Forward 5'-GCTGTCCCTGTATGCCTCTG-3'
Reverse 5'-GATGTCACGCACGATTTCCC-3'

α-*synuclein*
Forward 5'-CAGAGGCAGCTGGAAAGACA-3'
Reverse 5'-CACCACTGCTCCTCCAACAT-3'

TNF
Forward 5'-GAGCCAGCGCGCCAACGCCCTCCT-3'
Reverse 5'-TGAGGAGCACGTAGTCGGGGCAGC-3'

NF-κβ
Forward 5'-ACTGCCGAGCTCAAGATCTGCCGA-3'
Reverse 5'-AAGGAGCCTCGTGCCTCCCAGCCT-3'

qPCR

1 Extract total RNA and protein from cells or tissue using TRI Reagent® solution according to the manufacturer's instructions. Use RNase free glycogen as a carrier for the total RNA.
Keep protein for use in western blot.

2 Dissolve the RNA in nuclease free water.

3 Measure the concentration of RNA with a spectrophotometer (e.g. Nanodrop 1000).

4 Make cDNA from 2 micrograms of total RNA with a high-capacity cDNA reverse transcription kit (Invitrogen) according to manufacturer's instructions.

5 Dilute cDNA from the reverse transcription reaction 1:10 with nuclease free water.

6 Amplify 2 microliters of cDNA with gene specific intron-spanning primers (see materials) using PowerUp™ SYBR™ Green Master Mix (Invitrogen) according to the manufacturer's instructions with a StepOne Real-Time PCR system (Applied Biosciences).

7 Analyse using the using the comparative $2^{-\Delta\Delta}C_T$ method.