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 We use this protocol and it's working

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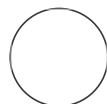
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🌐 qPCR of α -synuclein, TNF and NF- κ B

Michael J Hurley^{1,2}

¹Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, Maryland, USA



Michael J Hurley

ABSTRACT

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

MATERIALS

β -actin

Forward 5'-GCTGTCCCTGTATGCCTCTG-3'

Reverse 5'-GATGTCACGCACGATTTCCC-3'

α -synuclein

Forward 5'-CAGAGGCAGCTGGAAAGACA-3'

Reverse 5'-CACCCTGCTCCTCCAACAT-3'

TNF

Forward 5'-GAGCCAGCGCGCCAACGCCCTCCT-3'

Reverse 5'-TGAGGAGCACGTAGTCGGGGCAGC-3'

NF- κ B

Forward 5'-ACTGCCGAGCTCAAGATCTGCCGA-3'

Reverse 5'-AAGGAGCCTCGTGCCTCCAGCCT-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.