Apr 02, 2023

qPCR of α-synuclein, TNF and NF-κβ

DOI
dx.doi.org/10.17504/protocols.io.rm7vzb2z2vx1/v1

Michael J Hurley[1,2]

[1]Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK; [2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, Maryland, USA

Michael J Hurley
UCL

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzb2z2vx1/v1

Protocol Citation: Michael J Hurley 2023. qPCR of α-synuclein, TNF and NF-κβ . **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzb2z2vx1/v1

Protocol status: Working
We use this protocol and it's working

Created: February 27, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 77702

Keywords: ASAPCRN, synuclein, transcription pcr, κβ

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000420

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′

Troubleshooting

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.