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Quantitative RT-PCR



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Protocol status: Working

We use this protocol and it's working

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Abstract

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- 1 Isolate mRNA from iPSC-derived neurons, astrocytes, and microglia using the RNeasy Mini Kit (Cat. number 74106, QIAGEN, Germany) following the manufacturer's instructions.
- 2 Perform reverse transcription of the isolated RNA using the QuantiTect Reverse Transcription Kit (Cat. number 205313, QIAGEN) to synthesize cDNA.
- 3 Set up quantitative PCR reactions using SYBR Green PCR Master Mix (Cat. number 204145, QIAGEN).
- 4 Conduct qPCR in a Viia 7 Real-Time PCR System (Applied Biosystems, CA, USA).
- 5 Normalize the expression level of each target gene to the housekeeping genes ribosomal protein large P0 (RPLP0) or actin to account for RNA input variability.
- 6 Calculate relative gene expression levels using the $2^{-\Delta\Delta CT}$ method.
- 7 Determine fold changes in gene expression based on the calculated relative expression values.
- 8 Refer to Supplementary Table 2 of the study for the primer sequences used in the reactions.