May 08, 2025

Gene expression analysis

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DOI
https://dx.doi.org/10.17504/protocols.io.yxmvmywebv3p/v1

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Protocol Citation: Lucas Blasco-Agell, Meritxell Pons-Espinal, Jara Montero-Muñoz, Angel Raya, Antonella Consiglio, Miquel Vila 2025. Gene expression analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvmywebv3p/v1

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

Created: April 17, 2025

Last Modified: May 08, 2025

Protocol Integer ID: 130936

Keywords: human microglia cell, neuromelanin, gene expression, neurotoxic factor, gene expression analysis

Funders Acknowledgements:
ASAP
Grant ID: ASAP-020505

Abstract

To evaluate whether human microglia cells (hMG) can be activated by neuromelanin (NM), hMG were cultured with and without the presence of NM for 24 h and then harvested to measure gene expression of selected pro-inflammatory and neurotoxic factors.

1 *RNA* is extracted using a RNeasy® Mini kit (Qiagen) according to manufacturer's instructions and quantified with a NanoDrop one spectrophotometer (Thermo Fisher Scientific).

2 Reverse transcription into complementary cDNA is performed with the Superscript III First-Strand Synthesis System (Invitrogen™) to a final concentration of 2 ng/µL.

3 Gene expression is measured by real time quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR).

4 8 ng of cDNA are loaded in every well of a MicroAmp™ Optical 384-well Reaction Plate (Applied Biosystems™) with 10 nM of forward and reverse primers (Life Technologies) and PowerUp™ SYBR™ Green Master Mix (Applied Biosystems™).

5 Plate is run in a 7900HT Fast Real-Time PCR System with 384-well Block Module (Applied Biosystems™) following a standard cycling mode.

6 Cycling values are normalized to the housekeeping gene β-Actin and the relative fold gene expression of samples was calculated using the $2^{-\Delta\Delta Ct}$ method.