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Intracellular ROS measurement in iPSC-derived microglia V.1

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Irene Fernández-Carasa[1,2], Lucas Blasco-Agell[1,2], Jara Montero-Muñoz[1,2], Antonella Consiglio[1,2], Miquel Vila[3]

[1]Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital- IDIBELL, 08908 Hospitalet de Llobregat, Spain;
[2]Institute of Biomedicine of the University of Barcelona (IBUB), Carrer Baldiri Reixac 15-21, Barcelona 08028, Spain.;
[3]VHIR-CIBERNED-ASAP

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Miquel Vila
VHIR-CIBERNED-ASAP

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We use this protocol and it's working

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Abstract

Intracellular ROS measurement in iPSC-derived microglia

1 Intracellular ROS is measured in hMG exposed to 5 ng/mL NM for 24 hours using 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH2-DA) probe (Sigma-Aldrich), which turns highly fluorescent upon oxidation.

2 Ten minutes before adding the probe, hydrogen peroxide (1:25) is added to a well as a positive control of oxidation and absolute ethanol as negative control.

3 Then, 500 µM of DCFH2-DA are added for one hour at 37ºC. After that, cells are rinsed and recovered with a scrapper in PBS with 1% Triton.

4 Samples are placed in CellCarrier™-96 Ultra Microplate and fluorescence is measured within a FLUOstar Omega microplate reader (BMG LABTECH) at 485nm/530nm.

5 Results are normalized to protein concentration measured using a Micro BCATM Protein Assay Kit (Thermo Fisher Scientific).